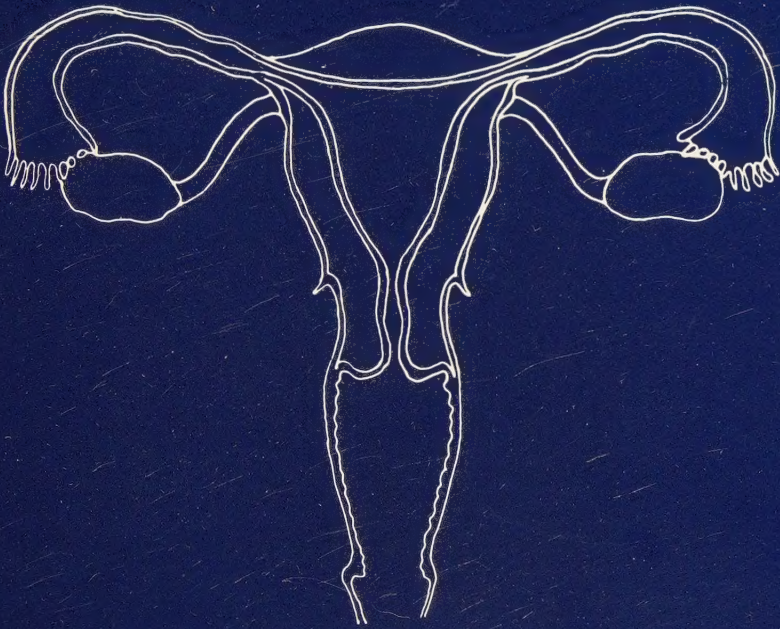
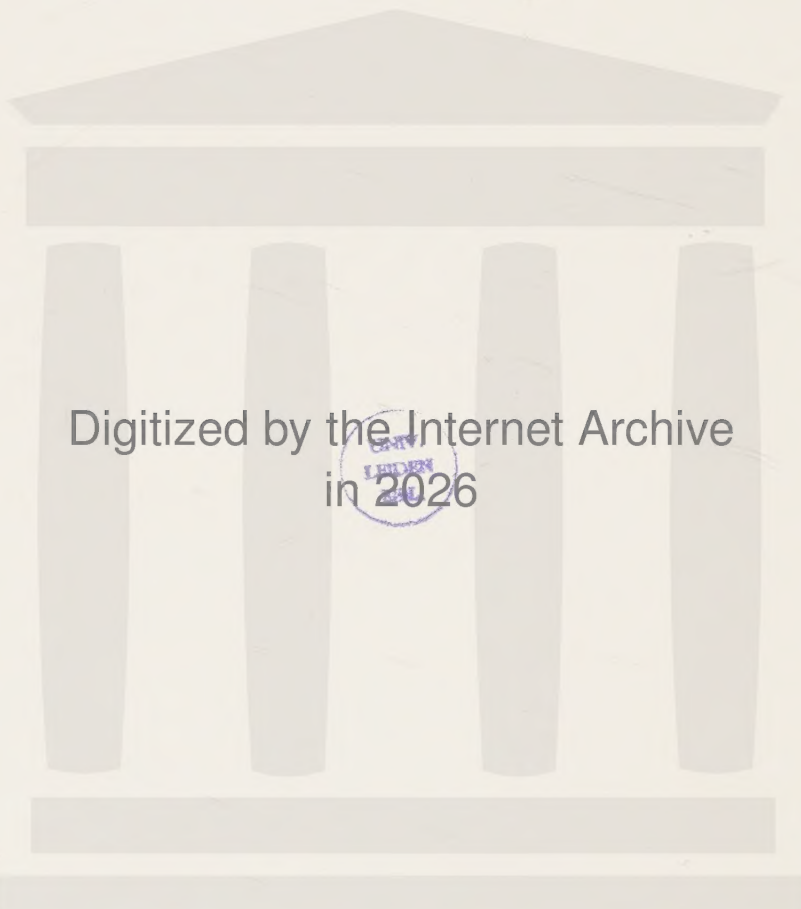


Histological aspects on a malignancy grading system for invasive squamous cell carcinoma of the uterine cervix

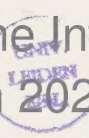
By Helena Willén



Lund 1984



Digitized by the Internet Archive
in 2026



HISTOLOGICAL ASPECTS ON A MALIGNANCY GRADING SYSTEM FOR INVASIVE
SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

av

Helena Willén

MUDr

leg.läkare

Lunds nation

Akademisk avhandling

som med vederbörligt tillstånd av Medicinska Fakulteten i Lund
för avläggande av medicine doktorsexamen kommer att offentligen
försvaras i Patologiska institutionens föreläsningssal,
Lasarettet i Lund, fredagen den 30 mars 1984, kl. 9.15.

Organization LUND UNIVERSITY Department of Pathology and Gynecologic Division of Department of Oncology, University Hospital of Lund, S-221 85 LUND		Document name DOCTORAL DISSERTATION	
		Date of issue February 1984	
		CODEN: LUMEDW/(MEPR-1004)/1-120 (1984)	
Author(s) HELENA WILLEN		Sponsoring organization	
Title and subtitle HISTOLOGICAL ASPECTS ON A MALIGNANCY GRADING SYSTEM FOR INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX			
Abstract Histological aspects on a malignancy grading system for invasive squamous cell carcinoma of the uterine cervix. A histopathologic malignancy grading system (MGS) has been used for invasive squamous cell carcinoma of the uterine cervix. <u>Four tumour-cell parameters; structure, cell type, nuclear polymorphism, mitoses and four tumour-host relationship parameters; mode of invasion, stage of invasion, vascular invasion and host-cellular response, were recorded separately.</u> Each parameter was defined and described in detail. Fixation in 10 per cent formalin and 0.17 molar phosphate buffer at pH 7.0 with an effective osmolar pressure of 350 mOsm/l gave optimal stainability and minimized tissue shrinkage and section artefacts. Vessels remained open, which facilitated the evaluation of vascular invasion. Study of the predictive value of the different cell types of invasive squamous cell carcinoma showed that large cell non-keratinizing tumours had the best prognosis. In combination with clinical staging according to FIGO, the differentiation into cell type has an additional predictive value only in stage II. The predictive value of the MGS with respect to the 10-year survival was tested by different statistical analyses. For cases in stages I A - II B the MGS showed a better prognostic capability than staging according to FIGO classification alone. The MGS was clearly the most powerful predictive factor, but it was possible to reduce the number of parameters to a partial index composed of vascular invasion and host-cellular response only, without substantial loss of predictive capacity.			
Key words Malignancy grading system, invasive squamous cell carcinoma, cervix uteri, histology.			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN	
Recipient's notes		Number of pages	Price
		Security classification	

Distribution by (name and address) Helena Willén, leg läkare, Department of Pathology, University Hospital of Lund, S-221 85 LUND, Sweden.

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Helena Willén

Date 1984-02-06

HISTOLOGICAL ASPECTS ON A MALIGNANCY GRADING SYSTEM FOR INVASIVE
SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX

By Helena Willén



DEPARTMENT OF PATHOLOGY AND GYNECOLOGIC DIVISION OF
DEPARTMENT OF ONCOLOGY, UNIVERSITY HOSPITAL, LUND, SWEDEN
LUND 1984

BLOMS BOKTRYCKERI AB, LUND 1984

Quemadmodum in orbe rerum nihil est otiosum, tantum ut sit,
omnia sunt prope aliquem finem et usum, ut agant aliquid et
alicui serviant: ita in orbe scientiarum nihil sit speculativum
tantum, ut sciatur, omnia tendant in opus et usum, ut prosint.

J.A. Comenius: Ex diario 3. Decembris 1646.

To Julie and Rudolf



ABSTRACT

Histological aspects on a malignancy grading system for invasive squamous cell carcinoma of the uterine cervix.

A histopathologic malignancy grading system (MGS) has been used for invasive squamous cell carcinoma of the uterine cervix. Four tumour-cell parameters; structure, cell type, nuclear polymorphism, mitoses and four tumour-host relationship parameters; mode of invasion, stage of invasion, vascular invasion and host-cellular response, were recorded separately. Each parameter was defined and described in detail. Fixation in 10 per cent formalin and 0.17 molar phosphate buffer at pH 7.0 with an effective osmolar pressure of 350 mOsm/l gave optimal stainability and minimized tissue shrinkage and section artefacts. Vessels remained open, which facilitated the evaluation of vascular invasion. Study of the predictive value of the different cell types of invasive squamous cell carcinoma showed that large cell non-keratinizing tumours had the best prognosis. In combination with clinical staging according to FIGO, the differentiation into cell type has an additional predictive value only in stage II. The predictive value of the MGS with respect to the 10-year survival was tested by different statistical analyses. For cases in stages I A - II B the MGS showed a better prognostic capability than staging according to FIGO classification alone. The MGS was clearly the most powerful predictive factor, but it was possible to reduce the number of parameters to a partial index composed of vascular invasion and host-cellular response only, without substantial loss of predictive capacity.

Apart from certain unpublished results the present work is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. Invasive squamous cell carcinoma of the uterine cervix.
I: Definition of parameters in a histopathological grading system. Ulf Stendahl, Helena Willén and Roger Willén.
Acta Radiol. Oncol. 19:(1980), 467-480.
- II. Invasive squamous cell carcinoma of the cervix uteri.
VII: Influence of vehicle osmolarity on biopsy quality.
Helena Willén, Roger Willén and Ulf Stendahl. Acta Radiol. Oncol. 22:(1983), 257-261.
- III. Invasive squamous cell carcinoma of the uterine cervix.
VI: Prediction value of non-keratinizing, parakeratotic and orthokeratotic cell forms and clinical staging.
Helena Willén, Roger Willén and Ulf Stendahl. Acta Radiol. Oncol. 21:(1982), 401-406.
- IV. Survival and malignancy grading of invasive squamous cell carcinoma of the uterine cervix treated by irradiation in Lund 1969-1970. Gunnar Eklund, Jan-Erik Johnsson, Ulf Stendahl, Claes Tropé and Helena Willén.
Accepted for publication Acta Radiol. Oncol. (1984).
- V. Invasive squamous cell carcinoma of the uterine cervix.
Reconstruction of a partial index in a histopathologic malignancy grading system. Helena Willén and Gunnar Eklund. Accepted for publication Acta Radiol. Oncol. (1984).

ABBREVIATIONS

FIGO: Fédération Internationale de Gynécologie et Obstétrique (1971):

Stage I: Carcinoma strictly confined to the cervix (extension to corpus should be disregarded).

Stage IA: Cases of early stromal invasion.

Stage IB: All other cases of stage I.

Stage IIA: Carcinoma involving the vagina, but not the lower third.

Stage IIB: Carcinoma extending beyond the cervix, but not the pelvic wall.

Stage III: Carcinoma extending to the pelvic wall, on rectal examination no tumour free space in between. The tumour involves the lower third of the vagina.

Stage IV: Carcinoma extending beyond the true pelvis or involving the mucosa of the bladder or rectum. A bullous oedema as such does not permit allotment of a case to stage IV.

MGS	Histopathologic Malignancy Grading System
P ₁	Structure
P ₂	Differentiation into cell type
P ₃	Nuclear polymorphism
P ₄	Mitoses
P ₅	Mode of invasion
P ₆	Stage of invasion
P ₇	Vascular invasion
P ₈	Cellular response (lympho-plasmocytic)
S/L	Survival/Lethality

CONTENTS

INTRODUCTION	9
AIM OF THE PRESENT STUDY	11
MATERIAL	12
METHODS	13
RESULTS	17
GENERAL DISCUSSION	25
GENERAL CONCLUSION	35
ACKNOWLEDGEMENTS	36
REFERENCES	37

- o -

PAPERS I - V	49
--------------	----

INTRODUCTION

The idea of selecting treatment modality and of predicting prognosis on the basis of histopathologic classification of malignant tumours is quite old. The intention of proposed classifications has been to predict the prognosis of individual patients with a fair degree of certainty. Most classifications have been monofactorial with varying, often directly conflicting results (STENDAHL et coll. 1980). Monofactorial systems based on cell differentiation have been used together with clinical staging, which for some time has proven to be of superior prognostic value.

The WHO (POULSEN et coll. 1975) adopted the histological classification of invasive squamous cell carcinoma of the uterine cervix proposed by Wentz and Reagan (1959) and Reagan and Wentz (1967). Invasive squamous cell carcinomas were divided into large cell non-keratinizing, large cell keratinizing and small cell carcinoma. Patients with large cell non-keratinizing carcinoma showed a significantly higher survival rate compared with large cell keratinizing or small cell tumours.

At the Department of Tumour Pathology of the Radiumhemmet, Stockholm, a malignancy grading system with 8 parameters, each graded from 1 to 4 points, was developed to evaluate the biologic activities of squamous cell carcinoma of the larynx (JAKOBSSON et coll. 1973, JAKOBSSON 1975), palate (ENEROTH & MOBERGER 1973) and modified for gingiva (WILLEN et coll. 1975). The same or modified system was used by LUND et coll. (1975 a, b, 1976, 1977) in carcinomas of the lip, tongue and larynx, and by FISHER (1975) and HELWEG-LARSEN et coll. (1978) for the larynx. It was also used for cancer of the vulva (KABULSKI et coll. 1978) and of the tongue (HOLM et coll. 1982).

The malignancy grading system (MGS) was adopted and modified by STENDAHL et coll. (1979) for squamous cell carcinoma of the uterine cervix. 8 parameters, but graded from 1 to 3 points, and

one new parameter (differentiation into cell type) were analysed. In a retrospective investigation a high correlation was found between the sum of malignancy points and the clinical outcome (STENDAHL et coll. 1979, 1981 a, 1983).

The intra- and inter-observer reproducibility of the MGS for invasive squamous cell carcinoma of the uterine cervix has been extensively discussed (STENDAHL et coll. 1981 c). SISSONS (1975) pointed out that diagnostic instability is dependent on several factors such as size of the biopsy, fixation artefacts, slide thickness etc. These factors, therefore, require standardization. Very few investigations have been made concerning fixation for conventional light microscopic examination (BEYER-BOON et coll. 1979, DAVENPORT & BALL 1979, RUITENBERG et coll. 1982). Cervical tissue consists of firm components such as muscular tissue, vessels and nerves in combination with epithelial cells having varying cohesion with the underlying stroma. Artefacts may occur because of suboptimal fixation or mechanical trauma. Vascular invasion has proved to be a particularly valuable prognostic parameter (BARBER et coll. 1978, FRIDELL et coll. 1967), the estimation of which is important, but dependent on biopsy quality (STENDAHL et coll. 1981 b, c). Nahhas et coll. 1983 and Roche and Norris 1975 cast doubt on the significance of vascular involvement, particularly because of the difficulties of proving that capillary-like spaces were, in fact, capillaries.

STENDAHL et coll. (1981 b) studied a data reduction problem for the MGS. A partial index composed of 4 items (mitoses, mode of invasion, cellular response and vascular invasion) only was constructed by regression analysis. Vascular invasion was attributed double weight. This index (range 5 - 15 points) showed a good prognostic capacity for patients with invasive squamous cell carcinoma of the uterine cervix stage II. The reliability of such a partial index should be tested in further studies to assure that the parameters chosen in the original studies as having a high predictive value were not favoured by chance.

AIM OF THE PRESENT STUDY

1. To give a definition and detailed description of each histopathologic MGS (malignancy grading system) parameter which was used in this study.
2. To determine the osmolarity of the fixative which is optimal for biopsies of squamous cell carcinoma of the uterine cervix.
3. To study the predictive value of the different cell types of invasive squamous cell carcinoma of the uterine cervix with respect to the 10-year lethality rate.
4. To examine survival and malignancy grading in invasive squamous cell carcinoma of the uterine cervix stage I A - II B treated by irradiation in Lund 1969 - 1970.
5. To consider the construction of a partial index and, if necessary, the proposal of a new index.
6. To investigate the possibilities for practical application of MGS in routine gynecological oncology.

MATERIAL

II.

15 cones from patients with carcinoma in situ and biopsies from 45 patients with invasive squamous cell carcinoma, stages I - IV, taken at the University Hospital of Lund, were studied.

III.

432 consecutive patients with stage I B - IV invasive squamous cell carcinoma of the uterine cervix FIGO classified and treated at the Department of Gynecologic Oncology, University Hospital, Uppsala, during the years 1967 to 1971 were studied. In the final analysis 3 patients were omitted following a revision in their diagnoses and 4 because of unsuitable biopsy material. Thirty-two patients died from intercurrent diseases. Thus 393 patients were included in the series. Their age varied from 22 to 82 years. Follow-up was at least 10 years.

IV.

208 patients with invasive squamous cell carcinoma of the uterine cervix, stages I A - II B, treated by irradiation at the gynecologic section, Department of Oncology, University Hospital of Lund during the period 1969 - 1970 were investigated. Ten patients who received less than 60 per cent of the intended irradiation dose in point A (JOHNSON 1977) were excluded. 30 patients who could not be evaluated with respect to all histologic parameters or had a biopsy of poor quality were also excluded, leaving 168 patients in the study. Correction was made for patients dead from intercurrent disease within 5 years after onset of disease (9 patients) $n = 159$. The mean age at admission was 49 years. The follow-up was 10 years.

V.

The same material with the same exclusion criteria as in paper IV. was studied. Patients who died from intercurrent disease within 4 years after the diagnosis of cancer were also excluded, leaving 161 patients in the study. The follow-up which was

available at least 10 years, but only the first 8 year period was taken into account. This was done to make possible a comparison with a previous study (STENDAHL et coll. 1981 b).

METHODS

MGS histopathology (I, IV, V)

The tumour cell population and the tumour host relationship were considered separately. The evaluation of the tumour cell population (Table 1) was based on the grading of structure (P_1), cell type (P_2), nuclear polymorphism (P_3) and the frequency of mitotic figures (P_4) in terms of a 1 - 3 point scale. The tumour host relationship (Table 2) was also estimated in terms of 1 to 3 points by the mode of invasion (P_5), stage of invasion (P_6), vascular invasion (P_7) and the degree of lymphoplasmocytic infiltration. These 8 morphologic parameters permitted a grading with 8 - 24 points totally. The original slides were used. All biopsies were fixed in neutral formalin, embedded in paraffin and cut at 5 μ m. Either van Gieson or hematoxylin-eosin stained slides were used depending on the routine technique used at the time of the original classification.

Tissue fixation (II)

The cone material was cut into 8 fractions and put into the following solutions for 24 hours: (A) 10% unbuffered formalin (1:10 diluted commercial formaldehyde) given a total osmolarity $\geq$ 1600 mmol/l and an effective osmolarity of $\leq$ 50 mmol/l, (B) 10% formalin in phosphate buffer pH 7.0, with a molarity of 0.05 (100), 0.09 (200), 0.12 (250), 0.15 (310), 0.17 (350), 0.20 (400) and 0.26 (510), respectively. Effective osmolarity within brackets (mmol/l). Two biopsies were taken from each patient with invasive squamous cell carcinoma. One was fixed in either 0.15 (310), 0.17 (350) or 0.26 (510) molar phosphate buffer, pH 7.0, with 10% formalin and the other in conventional 10% unbuffered formalin (<50). The material was then processed in a routine fashion and stained with hematoxylin-eosin, van Gieson

Table 1

Parameters used for malignancy point grading. Tumour cell population.

Para- meter number		Points		
		1	2	3
1	Structure	Exophytic, papillary and solid.	Small cords and groups of cells.	Marked cellular dissociation.
2	Cell type	Large cell, no keratinization.	Large cell keratinized.	Small cell, no keratinization.
3	Nuclear polymorphism	>75% mature nuclei, few enlarged nuclei.	75 to 25% mature nuclei, moderate number of enlarged nuclei.	<25% mature nuclei, numerous irregular or ana- plastic enlarged nuclei.
4	Mitoses	Single 0-1.	Moderate number 2-5.	Numerous >5

Table 2

Parameters used for malignancy point grading. Tumour-host relationship.

Parameter number	Points			
	1	2	3	
5	Mode of invasion	Well defined borderline.	Cords, less marked borderline.	Groups of cells or diffuse growth.
6	Stage of invasion	Min. stroma inv. or microcarcinoma.	Nodular into submucosa and connective tissue.	Massive; amongst muscles, and vessels.
7	Vascular invasion	None.	Possible.	Well established within the lumina of lymph or blood vessels.
8	Cellular response lympho-plasmocytic	Marked continuous rim.	Moderate several large patches.	Slight or none: few small patches or no cells.

stain for fibrous tissue, PAS for glycogen and neutral mucins, Gordon-Sweet procedure for reticular fibers and Sirius stain for basal membrane and fibrous tissue.

Histopathologic differentiation into cell type (III)

The definitions of REAGAN et coll. (1957) were carefully followed, apart from additional sub-grouping into parakeratotic and orthokeratotic large cell keratinizing forms, as follows:

Type 1: Large cell non-keratinizing squamous carcinoma:
Tumours having no keratin production or only single cells with intracytoplasmic keratin were referred to this group. A single superficial layer of parakeratosis can also be accepted in this group.

Type 2: Large cell keratinizing squamous carcinomas

2P Parakeratotic form: Tumours showing keratinization but not typical keratin pearl formation were included here. Keratinization is often confined to the superficial portions of the carcinoma and can be composed of several cell layers of varying numbers of preserved tumour cell nuclei.

2K Orthokeratotic form: All tumours with large light cells and keratin pearls of both orthokeratotic and parakeratotic forms were referred to this group as were tumours with several layers of orthokeratotic desquamating cells confined to the superficial area of the cancer.

Type 3: Small cell carcinoma. Small dark cells with a high nuclear/cytoplasmic ratio and orangophilic cytoplasm.

STATISTICS

Significance analysis was done in order to investigate significant correlations to survival (CHI-square-test or Fisher exact test) in papers III, IV, V. Estimation and significance testing in the regression analyses were made with the help of the program BMDP2R for linear regression stepwise analysis and for Cox regression program BMDP2L (DIXON 1981) in papers IV, V. AID (Automatic Interaction Detector) analysis was performed according to an Osiris package (OSIRIS III 1973, RATTENBURY &

PELLETIER 1974). Mass significance analysis was performed according to EKLUND and GAVATIN (1972) in paper V.

RESULTS

Paper I.

Invasive squamous cell carcinoma of the uterine cervix.
I. Definition of parameter in a histopathologic malignancy grading system.

A histopathologic malignancy grading system (MGS) for invasive squamous cell carcinoma of the uterine cervix was defined and described in detail for practical use in surgical pathology. Histological classifications of squamous cell carcinoma since 1912 were also briefly reviewed.

Paper II.

Invasive squamous cell carcinoma of the cervix uteri.
VII. Influence of vehicle osmolarity on biopsy quality.

The optimal vehicle osmolarity has been examined for both carcinoma in situ and invasive squamous cell carcinoma of the uterine cervix in order to facilitate the analysis of all parameters, especially vascular invasion. Both hypoosmolar and hyperosmolar vehicles have been tested. Hypoosmolar vehicle caused hypotonic damage consisting of rarefaction of cytoplasmic organelles, cracking and swelling distortions as well as collapse of small vessels. The tissue treated with hyperosmolar vehicle became stiff and shrunken with increased intercellular tissue spaces and was difficult to cut. The optimal fixative was 0.17 mol phosphate buffer at pH 7.0 with an effective vehicle osmolarity of 350 mmol. This fixative gave good stainability, with considerable decrease of tissue damage and artefact. The estimation of vascular invasion become easier because vessels remained open.

Paper III.

Invasive squamous cell carcinoma of the uterine cervix. VI. Prediction value of non-keratinizing, parakeratotic and orthokeratotic cell forms and clinical staging.

The histopathologic classification based on differentiation into cell type described by Reagan et coll. (1957) and Reagan & Wentz (1967) was used for a study of 393 patients with invasive squamous cell carcinoma of the uterine cervix stages I B - IV with respect to the 10-year survival. Tumours of the orthokeratotic cell type had the highest lethality rate and the large cell non-keratinizing tumours had the lowest. Cell type differentiation by itself had a prognostic value which was subordinate to that derived from clinical staging. In combination with clinical staging, the differentiation into cell type had no further predictive value except for patients in stage II.

Paper IV.

Survival and malignancy grading of invasive squamous cell carcinoma of the uterine cervix treated by irradiation in Lund 1969-1970.

168 patients with invasive squamous cell carcinoma of the uterine cervix stage I A - II B treated by radiotherapy during the period 1969-1970 have been followed up. The 10-year survival/lethality was correlated to the following multivariate prognostic factors: MGS, histological classification according to Ackerman & Del Regato and differentiation into cell type (Wentz & Reagan), patients age, admission year, treatment and clinical stage according to the FIGO classification.

There was a lower 10-year survival rate in stage II B compared to stage I A - I B ($p < 0.01$). There was no significant difference between age groups with respect to the 10-year survival. Highly differentiated carcinomas according to Ackerman & Del Regato showed a better 10-year prognosis than anaplastic ones ($p < 0.01$).

The MGS system was an excellent predictor of prognosis. Patients with a low point MGS score (≤ 12) all survived both 5- and 10-year follow-up. Even in the middle group 13-15 MGS points the survival was 90%. The MGS of high point score (16 and more points) was associated with a significantly poorer prognosis ($p < 0.001$).

Different items of the MGS were studied in relation to the 10-year survival. There was a very strong correlation between the vascular invasion (P_7) and host-cellular response (P_8) and survival ($p < 0.001$).

Multivariate analyses of the prognosis showed that the MGS was strongly correlated to the survival. When each factor was analyzed separately, several were found to have a significant correlation to the 10-year survival (for example vascular invasion P_7 , tumour-host cellular response P_8 and histological classification according to Ackerman & Del Regato and FIGO staging).

Multiple linear stepwise regression analysis, AID analysis and Cox regression analysis and logistic regression analysis have been performed in order to test if the correlation remains when different variables were analyzed simultaneously. If the MGS were included it became the dominating variable. If the MGS were excluded from the analysis, vascular invasion (P_7) was the strongest variable together with host-cellular response (P_8) followed by structure (P_1) and stage II B, which showed the highest regression coefficients. The FIGO staging, histological classification into cell type or according to Ackerman & Del Regato or other single MGS items did not have the same predictive value as the MGS.

The AID analysis could identify a low risk patient group with 96 per cent 10-year survival, patients in stage I A - II A or patients with ≤ 15 MGS score.

The relation between MGS and the proportional hazard rate during the 10-year follow-up was analyzed by Cox regression analysis. The Z variable was defined in order to illustrate the role of time-dependant change of the MGS on the survival. A positive Z variable implies that the proportional hazard rate will increase during the years after admission. The regression analysis, considering even the censored cases, included 168 patients. An additional analysis including the MGS alone, MGS and Z and vascular invasion (P_7) and tumour-host cellular response (P_8) was carried out. The analysis showed, that the MGS was the dominating variable. No other variable could reach significance level. The coefficient 0.54 was obtained for the MGS, disregarding the other variables and was strongly significant. The Cox regression analyses confirmed that the MGS is the dominating prognostic factor. A comparison between the correlation to S/L of the MGS and FIGO stages showed that the MGS had a better prognostic capacity than FIGO staging alone. The results were consistent with previous similar studies.

Paper V.

Invasive squamous cell carcinoma of the uterine cervix.
Reconstruction of a partial index in a histopathologic malignancy grading system.

The malignancy grading system (MGS) consisting of eight items was analyzed to determinate if a reduction in the number of items could be made without loss of predictive capacity. The reliability of a previously proposed partial index (Stendahl et coll. 1981 b) was also tested. The material consisted of 161 patients with invasive squamous cell carcinoma of the uterine cervix in stage I A - II B. The predictive values of the malignancy grading system, all single items and a reduced partial index were compared with each other with the aid of correlation and stepwise linear multiple regression analyses. The highest correlation to S/L was obtained from the MGS followed by vascular invasion (P_7), host-cellular response (P_8) and stage of invasion (P_6). All parameters, except

mitoses (P_4) showed a highly significant correlation to the MGS ($p < 0.001$). The correlations between the eight items were generally quite low (Table 3). The items of the tumour-cell population had no significant predictive value alone. The tumour-host relationship ($P_5 - P_8$) showed a considerably higher correlation to both S/L and the MGS. Vascular invasion (P_7) followed by host-cellular response (P_8) showed a strong significance, $p < 0.001$, in a multiple stepwise linear regression analysis. The partial index based on a combination of two items only (2-6 points), vascular invasion and host-cellular response ($P_7 + P_8$), could identify low and high risk patients as reliably as FIGO clinical staging especially for high risk patients. The MGS remains the most complete prognostic classification for invasive squamous cell carcinoma of the uterine cervix but a comparable prognostic reliability can be obtained from a partial index composed of only two items (vascular invasion and host-cellular response).

Ten untreated or uncomplete treated cases were excluded. The limit was set at 60 per cent of the estimated irradiation dose in order to eliminate patients with expected unfavourable prognosis. No attention was otherwise paid to the tumour dose. 7 of these patients were dead at the 10-year follow up. 6 of them died during the first 5 years. The majority of these tumours showed ≤ 15 MGS score.

A comparison between primary (d) and recurrence (r) biopsies with respect to MGS could be made in only 7 patients (Table 4). This group is very small, but it is striking that MGS scores of recurrence remain in the same MGS score group (low, middle and high). All of these cases had a partial index of three or more points.

In the present study cancer recurrence and spread was observed in 31 cases. No constant pattern of spread could be identified, but 13 of these patients belonged to the middle MGS group and 18 cases had a high MGS score. None of the patients belonged to the

Table 3

Correlation coefficients, r , between the eight items included in the MGS.

	P1	P2	P3	P4	P5	P6	P7	P8
P1	1.00							
P2	.19	1.00						
P3	.00	-.04	1.00					
P4	-.28	.06	-.16	1.00				
P5	.07	.10	.05	-.10	1.00			
P6	.16	.15	.03	.08	.22	1.00		
P7	.10	.19	.03	.01	.22	.25	1.00	
P8	.26	.03	.01	-.29	.00	.21	.17	1.00

Significance: $p < 0.05$ corresponds to $r \geq 0.16$

$p < 0.01$ " " $r \geq 0.20$

$p < 0.001$ " " $r \geq 0.26$

Table 4

Comparison between primary diagnosis (d) and recurrence (r) biopsies with respect to malignancy point values in 7 patients.

	diagnose/recurrence									
	d	r	d	r	d	r	d	r	d	r
P1 Structure	2	2	2	2	2	1	2	2	2	2
P2 Cell differentiation	3	3	2	2	1	1	1	1	2	2
P3 Nuclear polymorphism	3	3	3	3	1	2	3	3	3	3
P4 Mitoses	1	1	3	3	1	2	2	1	3	2
P5 Mode of invasion	3	3	1	2	2	2	1	3	2	3
P6 Stage of invasion	3	3	3	3	3	3	3	3	3	3
P7 Vascular invasion	3	2	1	1	3	1	1	1	2	2
P8 Host-cellular response	3	3	3	3	2	3	2	3	1	3
Total score	21	20	18	19	15	15	15	17	17	19
Partial index	6	5	4	4	5	4	3	4	3	5

low MGS group. 7 women had cancer growth in the portio and pelvis and distant metastases appeared in 10 cases (8 cases with distant metastases only, 2 with both distant metastases and local or pelvic tumour growth). Other sites were represented by a single case or up to three cases (portio alone, vagina). The majority (25 of 31) of the patients with recurrence and generalized disease had a partial index of three or more points. Only six patients had a partial index of 2 points (Table 5). A comparison between the partial index and complete MGS scores illustrates the better prognostic capability of the MGS (Table 6).

Table 5

Partial index	2	3	4	5	6	Total
Number of patients	6	5	7	8	5	31

Table 6

MGS score	8 - 12 p	13 - 15 p	16 - 24 p	Total
Number of patients	0	13	18	31

Distribution of cases with recurrence and disease with respect to Partial index (Table 5) and MGS (Table 6).

GENERAL DISCUSSION

The interpretation of histologic data has become more complex in recent years as clinicians demand more detailed histopathologic classification in order to select the more specialized and often individualized treatments now available. The present aim of clinical-pathologic cooperation will be to find diagnostic systems which will make possible the prediction of the clinical outcome for an individual patient. Up to now the best tool for the prediction of the course of invasive squamous cell carcinoma of the uterine cervix has been considered to be adequate clinical staging according to FIGO rather than histopathology.

Several multifactorial histopathological classification systems have been developed (for review see Stendahl et coll. 1980). These have been complicated, using up to 20 different factors. Classification into various subtypes of cancer and grading of premalignant or malignant lesions showed low reproducibility best expressed in terms of considerable inter-observer and intra-observer variation (Stendahl et coll. 1981 c, Collan & Romppanen 1982). The evaluation of a system with too many parameters and too detailed scores easily becomes rather subjective, making the system unsuitable for routine use.

The present MGS classification was based upon 8 parameters, evaluating the tumour-cell population and the tumour-host relationship separately (Stendahl et coll. 1980, Eklund et coll. 1984). In using a malignancy grading system, it is essential that all parameters can be identified in the primary biopsy. The biopsy should be large and adequately fixed in order to reduce the sampling errors. Some authors consider cervical biopsy to be inadequate as diagnostic material (Larsson et coll. 1983). The registration of MGS score can be difficult in retrospective studies because of poor biopsy quality and incomplete or uncertain registration of one or several items. In the present study (Eklund et coll. 1984), the original 198 cases had to be reduced to 168 cases ($\approx 16\%$) because of uncomplete

registration of one or several items. Items for the tumour-host relationship, especially stage of invasion and vascular invasion were often missing. As the most important parameters were lacking, it was considered inadequate to base the MGS on remaining items. The loss of cases due to poor biopsy quality could be reduced by an improved fixation and biopsy technique used in our prospective study of invasive squamous cell carcinoma of the uterine cervix (to be published).

The major aim of this study was to investigate the prospects of a practical application of MGS in routine gynecological oncology. From the results of the study we have found it possible to propose a reconsideration of the choice of MGS items.

Tumour cell population items showed a weak correlation to S/L. The possibility of a relationship between structure (P_1) and mode of invasion (P_5) has been discussed. In our material both parameters showed a highly significant correlation to MGS ($p < 0.001$) and were independent of each other.

Differentiation into cell type (P_2) according to Reagan et coll. and Reagan and Wentz (1957, 1967) was adopted by WHO (Poulsen et coll. 1975) in an attempt to achieve a standardized nomenclature of malignant cervical neoplasms. Patients with large cell non-keratinizing tumours had the best prognosis. Why keratinizing carcinomas seem to have a worse prognosis in patients treated by irradiation is unclear (Gynning et coll. 1983), but two explanations have been advanced. The first is that a real biologic difference between keratinizing and non-keratinizing tumours exists (Reagan et coll. 1957, Reagan & Wentz 1967) with differing cells of origin and tumour localisation (Wentz 1966, van Nagell et coll. 1977). According to Adams (1976), however, the question of keratinization in these tumours is quantitative rather than qualitative with separate cell types. According to van Nagell et coll. (1977), the incidence of lymph node metastases and tumour recurrence was

more directly related to tumour size than to tumour cell type. The second explanation is that the difference in prognosis is a function of radiosensitivity and that older reports were based on series which were inadequately treated. When patients are treated according to modern radiation techniques, no differences in treatment results between large cell non-keratinized and large cell keratinized carcinomas are seen (Gundersen et coll. 1974). Large cell keratinizing tumours showed significantly better prognosis in surgically treated patients (Larsson et coll. 1983). In our study, cell type differentiation by itself had a prognostic value which was subordinate to that derived from clinical staging (Willén et coll. 1982).

Small cell carcinoma showed a poor prognosis. It has been suggested that small cell carcinoma represents "either the least differentiated squamous carcinoma or may have a different tissue origin" (Wentz & Reagan 1959). Light and electron microscopic studies on these tumours have shown some features of carcinoids (Tateishi et coll. 1975, Albores-Saavedra et coll. 1976). Radiotherapy is considered inadequate in the treatment of carcinoid tumours (Albores-Saavedra et coll. 1976). The small cell carcinomas of the uterine cervix are rare but they probably should be excluded from squamous cell carcinoma classifications.

For nuclear polymorphism evaluation a modification of Broder's system (1920, 1925, 1926, 1940) was used. Per cent evaluation of mature nuclei is very subjective and results are difficult or impossible to reproduce (Stendahl et coll. 1981 c). Experience does not guarantee better reproducibility or accuracy. The reliability of histopathology can be improved by morphometry. Morphometry is or can be made more reproducible than subjective evaluation with microscope, eye and brain only. Morphometry should not be considered as a method which competes with other methods of histopathological investigation; it is a method for further exploiting them (Collan & Pomppanen 1982).

Morphometric methods have been specifically applied to normal and pathological stratified squamous epithelium (White et coll. 1980, Boysen & Reith 1980). Boysen and Reith studied metaplastic, dysplastic and carcinomatous alterations of the nasal mucosa in nickel workers. There was some increase in the size of the basal cells in metaplastic epithelium, whereas there was a slightly greater increase in dysplasia and a pronounced increase in carcinoma.

Baak et coll. (1982) used morphometric methods in the prediction of the clinical course for individual patients with carcinoma of the breast. Measurement of tumour diameter, assessment of mitotic and cellular indices and quantitative microscopy of nuclear features were assessed together with nuclear features and histological grades. There were reproducible significant differences between survivors and non-survivors. The most important parameters were cellularity index and mitotic activity index, followed by quantitative microscopical nuclear parameters and nuclear histological grade. By discriminant analysis of the quantitative microscopical data alone 82% of all patients could be correctly classified as survivor or non-survivor. Morphometry thus seems capable of prediction outcome in individual patients more accurately than usual staging/grading methods. Similar results were reported by Stenkivist et coll. (1981 a).

The relationship between nuclear volume and DNA content of cells in normal epithelium, or carcinoma in situ and of invasive carcinoma of the uterine cervix has been investigated (Valeri et coll. 1967). It was shown that cells of carcinoma of the uterine cervix constitute a heterogenous population. This heterogeneity varied from case to case, partially due to differences in DNA-content and nuclear volume. The changes in the ratio between nuclear volume and DNA-content in cells of carcinoma in situ of the uterine cervix tend to have values equivalent to those of invasive carcinoma.

Longmore and Cowpe (1982) studied the Feulgen DNA-content and nuclear area for both normal and pathologic oral squamous cells to assess the diagnostic potential of nuclear area determination. Nuclear area was increased significantly in malignant lesions but was also elevated in lesions showing no malignancy. Abnormal DNA profiles were detected in carcinomas and in one case showing mild epithelial atypia. They conclude that the determination of nuclear area has, at present, no specific diagnostic value since there appeared to be no clear relationship between nuclear area, DNA-content, clinical presentation and histopathology in abnormal cases. Similar results were reported by Nødskov-Pedersen (1971). In both studies microspectrophotometry was used.

Jakobsen et coll. (1979 a, b, 1983) studied the correlation of DNA-distribution and cytological differentiation of human cervical carcinomas by flow cytometry. They could demonstrate that well differentiated squamous cell carcinomas contained hyperploid cells predominantly with hyperdiploid DNA-content. Hyperploid cell populations in the moderately differentiated tumours are mainly in the hyperdiploid and tetraploid regions. Poorly differentiated tumours contain hypertetraploid and aneuploid cell populations. The results indicate that DNA distribution is correlated with cellular differentiation, but not with clinical stage. DNA distribution and cellular differentiation bear no relation to the extent of the disease, but these parameters might well be of prognostic value.

In the present study nuclear polymorphism showed fairly low correlation to the S/L (Willén & Eklund 1984). This can probably be improved by using morphometry but up to now it has been difficult to introduce morphometry investigation into routine surgical pathology.

DNA analysis by flow cytometry of our prospective material of almost 120 cases of invasive squamous cell carcinoma of the uterine cervix is in progress. The DNA-distribution could be

studied in most of the cases (to be published). This method can also be used in clinical practice.

Similar estimation and reproducibility problems are also present in the mitoses (P_4) item, where, again, some improvement in reproducibility could be expected by the use of morphometry or cytometry (Baak et coll. 1982, Stenkvis et coll. 1981 b). In our study mitoses (P_4) had no prognostic value by itself (Willén & Eklund 1984). There are many factors which influence the frequency of mitotic figures; fixation, section thickness etc (i.e. Sissons 1975). On the other hand Reagan and Fu (1979) pointed out that mitoses are common in large cell non-keratinizing carcinoma of the uterine cervix (which has the best prognosis in patients treated by irradiation) and uncommon in keratinizing carcinoma. Tumours with a high mitotic frequency (possibly with a higher malignancy potential) may show a higher sensibility for an irradiation treatment. Such counter-acting factors can be a part of the explanation.

In this study, parameters relating to the tumour-host relationship showed a strong correlation to S/L and MGS. Mode of invasion (P_5) is related to damage to the basement membrane as well as to the MGS score and stage of the disease. Ultra-structural study reveals that the normal basement membrane, both homogenous and microfibrillar, is separated from the cell by a lamina lucida and joined by anchoring fibrils to the surrounding connective tissue. Frappart et coll. (1982) studied basement membrane in the uterine cervix in its normal state and in pre-cancerous and cancerous states utilizing specific antibodies to the different types of collagen. The atypical surface epithelium and certain invasive squamous cancer lobules were surrounded by a discontinuous immunoreactive basement membrane of collagen type IV of uneven thickness. The membranes could also be demonstrated by PAS-staining. Similar results were shown by Rubio and Biberfeld (1975, 1979) and Rubio et coll. (1978). Cullhed (1978) believes that marked cell dissociation is probably caused by severe disturbances in the structure of the cell membrane which

carries with it intercellular disturbances. He found significantly higher occurrence of metastases (17.9%) with these tumours than in tumours with less dissociation (3%). Baltzer et coll. (1982 b) observed a 66.2% 5-year survival among patients with tumours showing pronounced dissociation and an 86.4% survival in patients with more cohesive carcinomas.

Stage of invasion (P_6) is by many authors expressed as invasion in mm. Poulsen et coll. (1975) defined microcarcinoma as stromal invasion limited to isolated microscopic foci (generally 5 mm or less in depth). Invasion depth is related to the lesion size which is considered to be the most accurate predictor of disease free survival (Shingleton 1983). Other authors could not find a prognostic significance for tumour volume (Baltzer et coll. 1982 a) or depth and lateral extension of tumour (Larsson et coll. 1983).

In routine biopsy material of invasive squamous cell carcinoma it is often difficult to reliably identify the surface or the basement membrane as a guide line for the measurement of the depth of invasion.

For an estimation of the stage of invasion we find it more convenient to note tumour-growth in relation to the medium-sized vessels, which identifies better the anatomical localization of the tumour infiltration than depth in mm.

Vascular invasion (P_7) and lymphoplasmocytic cell response (P_8) were the most important predictive parameters in this study (Eklund et coll. 1984, Willén & Eklund 1984). The partial index based on a combination of P_7 and P_8 (2-6 points), could identify low and high risk patients. Comparison between survival for the partial index had almost the same predictive power as the MGS.

The significance of vascular invasion and lymphocytic infiltration in invasive cervical cancer has been reported by several

authors (i.e. van Nagell et coll. 1978, Barber et coll. 1978, Stendahl et coll. 1982 b). Nahhas et coll. (1983) could not find vascular involvement to be of significance in itself.

They comment on the increased frequency of vascular involvement reported in the reevaluated slides compared to the original biopsy reports. There is no doubt that the main difficulty in the evaluation of vascular invasion is a proper identification of vascular channels. The study of the influence of vehicle osmolarity on biopsy quality indicates that an analysis of vascular invasion can be improved by better fixation of the surgical specimen (Willén et coll. 1983).

van Nagell et coll. (1978) observed that vascular invasion was only minimally related to lesion size and was associated with an increase in tumour recurrence particularly to extrapelvic sites. A marked lymphocytic response was unrelated to lymph node morphology, site of recurrence or incidence of lymph node metastases. Baltzer et coll (1982 b) noted better survival in patients with a heavy inflammatory infiltration in the region of the invasion front. Stromal eosinophilia alone is probably not related to survival (Bostrom & Hart 1981, Linnell & Månsson 1954).

A survey of tumour marker (CEA) in patients with squamous cell carcinoma of the uterine cervix gave a good result in patients with stage III disease (Fritsche et coll. 1982). van Nagell et coll. (1982) reported that CEA (carcinoembryonic antigen) content is related to cell type, being present in 82% of keratinizing squamous carcinomas but only in 50% of non-keratinizing tumours in both primary and metastatic cervical tumours. te Velde et coll. (1982) studied pretreatment CEA levels. Elevated pretreatment CEA levels, encountered in about 40% of all patients who developed recurrences, mainly reflected the ultimate, fatal prognosis and not so much the stage of the tumour or the site of recurrence. They conclude that, although CEA determination in cervical cancer may seem to provide useful

information with regard to the prognosis before treatment and the development of recurrences during follow-up, its clinical use is unlikely to have any impact on the incidence of cures obtained in the absence of appropriate chemotherapy.

Stenkvist et coll. (1982) studied time dependency in the predictive power of the variables predicting breast cancer recurrence. There were different variable combinations predicting the recurrence rate during the first 2.5 year period (size of axillary metastases and primary tumour, number of lymphocytes around the tumour, mitotic frequency and degree of differentiation) compared with the second 2.5 year period (variance of DNA content among tumour cell nuclei, number of lymphocytes around the tumour, occurrence of multiple tumours in the operated breast and occurrence of breast cancer among relatives). The authors emphasize, that the study of recurrence prediction in human breast should be based on an optimal combination of a number of variables which, independently, influence the prognosis.

Stendahl et coll. (1982) studied 25 cases of late local recurrence after radiation therapy. The mean value of the tumour-cell score did not differ significantly between the two estimates: 7.4 and 7.1 respectively. The mean value of the tumour-host relationship increased, however, from 7.9 to 9.6 points. In patients with 16 points or more there was no significant change between estimation. In patients scoring less than 16 points a half of patients had a significant increase in tumour-host score indicating the possibility of the occurrence of new prognostic factors.

In the present study (Eklund et coll. 1984), the prognostic significance of the MGS over time was analyzed with the aid of Cox proportional hazard regression model. It was confirmed that the MGS was the dominating prognostic factor, without loss of importance with time.

As the above discussion shows, the results of histological classifications or evaluation of prognostic parameters are quite often conflicting. As recently as 1983 it was stated that survival and recurrence in patients with cervical cancer are not a function of the tumour histology (Shingleton et coll).

The present investigation clearly indicates the potential of histopathology in the prediction of prognosis but it is evident that a monofactorial classification is not adequate for the enormously complex task of prognosis prediction. A multi-factorial MGS can, however, express the biologic activity of a tumour in any given patient. The knowledge of the predictive potential of the MGS should be used in treatment planning, especially in patients with high MGS scores. More specialized treatment modalities such as a combination of irradiation, surgery and cytotoxic drugs should be developed for this group of patients. The MGS can be recommended for practical use under the following conditions:

1. For proper evaluation the biopsy specimen must be of adequate size, optimally fixed and well processed and stained.
2. The evaluation must be done only by a pathologist well trained in MGS classification.
3. There must be a very good cooperation between the classifying pathologist and the clinician responsible for treatment. Each case must be individually analyzed and treated according to a standardized treatment program.

GENERAL CONCLUSIONS

1. Optimal fixation for biopsies with invasive squamous cell carcinoma of the uterine cervix was 10 per cent formalin and 0.17 mol phosphate buffer at pH 7.0 with an effective vehicle osmolarity of 350 mmol/l.
2. Large cell non-keratinizing squamous cell carcinomas of the uterine cervix had the best prognosis in patients treated by irradiation.
3. Differentiation into cell type itself had a prognostic value which is subordinate to that from clinical staging according to FIGO.
4. The malignancy grading system (MGS) consisting of eight items is a more powerful predictive tool for squamous cell carcinoma of the uterine cervix stage I A - II B than staging according to FIGO alone.
5. Patients with a low MGS score had an extremely good survival rate at both 5- and 10-year controls.
6. The items related to the tumour-host relationship give the most important prognostic information.
7. Partial index (range 2 to 6 points) composed only of vascular invasion (P_7) and host-cellular response (P_8) parameters showed a predictive capacity which is comparable to that for the complete MGS.
8. The MGS classification can be used in routine clinical and histopathological practice under certain given conditions.

ACKNOWLEDGEMENTS

I wish to express my thanks to all persons who have helped me during this study, and particularly to acknowledge:

Associate Professor Roger Willén, my tutor, for his continuous support and never failing encouragement;

Professor Gunnar Eklund for his remarkable tact and diplomacy and outstanding medical statistics;

Associate Professor Claes Tropé, my tutor in gynecologic oncology, for his encouragement, pleasant cooperation and for his support;

Professor Unne Stenram and Associate Professor John-Erik Johnsson for their cooperation, valuable constructive criticism and positive guidance;

Devoted friend, Doctor Ulf Stendahl for his cooperation;

Professor Nils O Berg for his encouragement, constructive criticism and interest in the study;

Doctor Robert Cameron for revising my English and for his friendship;

Ms Gun Kungberg, Ms Inga-Britt Carlsson and Ms Carina Johansson for excellent secretarial assistance;

The staff of the Department of Pathology for their generous help; particularly Mr Roger Lundholm for his photography.

Financial support was given by Stockholms Cancerföreningen, Stockholm, University Hospital and Medical Faculty of the University of Lund and John and Augusta Persson Foundation.

REFERENCES

ACKERMAN L.V and DEL REGATO J.A: Cancer diagnosis, treatment and prognosis. First edition C.V. Mosby, St Louis, 1947.

ADAMS D.: Keratinizing of the oral epithelium. Ann. Roy. Coll. Surg. Engl. 58:(1976), 351.

ALBORES-SAAVEDRA J., LARRAZA O., POUCELL S. and RODRÍGUES MARTÍNEZ H.A: Carcinoid of the uterine cervix. Additional observations on a new tumor entity. Cancer 38:(1976), 2328.

ARSLAN PAGNINI C., DELLA PALMA P. and De LAURENTIIS G.: Malignancy grading in squamous carcinoma of uterine cervix treated by surgery. Br.J.Cancer 41:(1981),415.

BAAK J.P.A., KURVER P.H.J., DE SNOO-NIEWLAAT A.J.E., DE GRAEF S., MAKKINK B. and BOON.M.E.: Prognostic indicators in breast cancer-morphometric methods. Histopathology 6:(1982), 327.

BALTZER J., KÖEPKE W., LOHE K.J., OBER K.G. and ZANDER J.: (a): Age and 5-year survival rates in patients with operated carcinoma of the cervix. Gynecol. Oncol. 14:(1982), 220.

BALTZER J., LOHE K.J., KÖPCKE W. and ZANDER J.: (b): Histological criteria for the prognosis in patients with operated squamous cell carcinoma of the cervix. Gynecol. Oncol. 13:(1982), 184.

BARBER H.R.K., SOMMERS S.C., ROTTERDAM H. and KWON T.: Vascular invasion as a prognostic factor in stage Ib cancer of the cervix. Obstet. Gynecol. 52:(1978), 343.

- BEYER-BOON M.E., VAN DER VOORN-DEN HOLLANDER M.J.A.,
ARENTZ P.W., CORNELISSE C.J., SCHABERG A. and FOX C.H.: Effect
of various routine cytopreparatory techniques on normal
urothelial cells and their nuclei. Acta path. microbiol. scand.
sect. A 87:(1979), 63.
- BOSTROM S.G. and HART W.R.: Carcinomas of the cervix with
intense stromal eosinophilia. Cancer 47:(1981), 2887.
- BOYSEN M., and REITH A.: A morphometric model for light
microscopic analysis of metaplastic, dysplastic and
carcinomatous alteration of the nasal mucosa in nickel workers.
Path.Res.Pract. 166:(1980), 362.
- BRODERS A.C.: Squamous cell epithelioma of the lip. JAMA
74:(1920), 656.
- BRODERS A.C.: The grading of carcinoma. Minn. Med. 8:(1925),
726.
- BRODERS A.C.: Carcinoma grading and practical application. Arch.
Path. (Chic) 2:(1926), 376.
- BRODERS A.C.: Microscopic grading in cancer. In: Treatment of
cancer and allied diseases, p.9. Edited by G.T.Pack and
E.M.Livingstone. Paul B. Hoeber, New York 1940.
- COLLAN Y. and ROMPPANEN T. (editors): Morphometry in
morphological diagnosis. Symposium on morphometry in
morphological diagnosis. Kuopio University Press, Kuopio, 1982.
- CULLHED S.: Carcinoma cervicis uteri stages I and IIa.
Treatmenthistopathology-prognosis. Thesis. Linköping 1978.

DAVENPORT Jr W.D. and BALL C.R.: Observations on the results of specific histochemical techniques and empirical staining methods on several tissue/organ types using a variety of fixing fluids. *Histopathology* 3:(1979), 321.

DIXON W.J. (editor): BMDP. Statistical Software, University of California Press, Berkley, Calif, 1981.

EKLUND G. and GAVATIN A.: Automatic interaction detector (AID) analysis. In: On the role of viruses in acute infectious diseases of the central nervous system. Edited by B.Sköldberg, *Scand. J. Infect. Dis.* (1972). Suppl. no. 3, p.89.

EKLUND G., JOHNSON J.-E., STENDAHL U., TROPE C. and WILLEN H: Survival and malignancy grading of invasive squamous cell carcinoma of the uterine cervix treated by irradiation in Lund 1969-1970. *Acta Radiol. Oncology* (accepted for publication 1984).

ENEROTH C.M. and MOBERGER G.: Histological malignancy grading of squamous cell carcinoma of the palate. *Acta Otolaryngol.* 75: (1973), 293.

FIGO-Fédération Internationale de Gynécologie et Obstétrique: Classification and staging of malignant tumours in female pelvis. *Acta Obstet. Gynecol. Scand.* 50:(1971), 1.

FISHER H.R.: Grading of biopsies of laryngeal carcinomas by multiple criteria. *Canad. J. Otolaryngol* 4:(1975), 881.

FRAPPART L., BERGER G., GRIMAUD J.A., CHEVALIER M., BREMOND A., ROCHET Y. and FEROLDI J.: Basement membrane of the uterine cervix: Immunofluorescence characteristics of the collagen component in normal or atypical epithelium and invasive carcinoma. *Gynecol. Oncol.* 13:(1982), 58.

FRIDELL G.H., STEINER G. and KISTNER R.W.: Prognostic value of blood-vessel invasion in cervical cancer. *Obstet. Gynecol.* 29:(1967), 855.

FRITSCHÉ H.A., FREEDMAN R.S., LIU F., ACOMB L.D. and COLLINSWORTH W.L.: A survey of tumor markers in patients with squamous cell carcinoma of the uterine cervix. *Gynecol. Oncol.* 14:(1982), 230.

GUNDERSEN L.L., WEEMS W.S., HEBERTSON R.M. and PLENK H.P.: Correlation of histopathology with clinical results following radiation therapy for carcinoma of the cervix. *Am. J. Roentgenol.* 120:(1974), 74.

GYNNING I., JOHNSON J-E, ALM P. and TROPE C.: Age and prognosis in stage Ib squamous cell carcinoma of the uterine cervix. *Gynecol. Oncol.* 15:(1983), 18.

HELWEG-LARSEN K., GRAEM N., MEISTRUP-LARSEN K.I. and MEISTRUP-LARSEN U.: Clinical relevance of histological grading of cancer of the larynx. *Acta path. microbiol. scand. Sect A.* 86:(1978), 499.

HOLM L.E., LUNDQUIST P.G., SILFVERSWÄRD C. and SOBIN A.: Histological grading of malignancy in squamous cell carcinomas of the oral tongue. *Acta Otolaryngol.* 94:(1982), 185.

JAKOBSEN A., BICHEL P. and SELL A.: (a): DNA distribution in biopsy specimens from human cervical carcinoma investigated by flow cytometry. *Virchows Arch. B Cell Path.* 29:(1979), 337.

JAKOBSEN A., BICHEL P. and SELL A.: (b): Correlation of DNA distribution and cytological differentiation of human cervical carcinomas. *Virchows Arch. B Cell Path.* 31:(1979), 75.

JAKOBSEN A., VANG NIELSEN K. and RONNE M.: DNA distribution and chromosome number in human cervical carcinoma. Anal. Quant. Cytol. 5:(1983), 13.

JAKOBSSON P.Å.: Histological grading of malignancy and prognosis in glottic carcinoma of the larynx. Canad. J. Otolaryngol. 4:(1975), 885.

JAKOBSSON P.Å., ENEROTH C.M., KILLANDER D., MOBERGER G. and MÄRTENSSON B.: Histological classification and grading of malignancy in cancer of the larynx (a pilot study). Acta Radiol. Ther. Phys. Biol. 12:(1973), 1.

JOHNSSON J-E.: Squamous cell carcinoma of the uterine cervix. Acta Radiol. Ther. Phys. Biol. 16:(1977), 33.

KABULSKI Z. and FRANKMAN O.: Histologic malignancy grading in invasive squamous cell carcinoma of the vulva. Int. J. Gynaecol. Obstet. 16:(1978), 233.

LARSSON G., ALM P., GULLBERG B. and GRUNSELL H.: Prognostic factors in early invasive carcinoma of the uterine cervix. A clinical, histopathologic and statistical analysis of 343 cases. Am. J. Obstet. Gynecol. 146:(1983), 145.

LINELL F. and MÅNSSON B.: The prognostic value of eosinophilic leukocytes in the stroma of carcinoma of the cervix uteri: A study of 291 cases treated with Radium and Roentgen therapy. Acta Radiol. 41:(1954), 453.

LONGMORE B.R. and COWPE J.: Nuclear area and Feulgen DNA content of normal and abnormal oral squames. Anal. Quant. Cytol. 4: (1982), 35.

LUND C., JØRGENSEN K., ELBRØND O., HJELM-HANSEN M. and ANDERSEN A.P.: Histologic grading of epidermoid carcinomas in the head and neck. Danish Med. Bull. 24:(1977), 162.

LUND C., SØGAARD H., ELBRØND O., JØRGENSEN K. and ANDERSEN A.P.
(a): Epidermoid carcinoma of the lip. Histologic grading in the
clinical evaluation. Acta Radiol. Ther. Phys. Biol. 14:(1975),
465.

LUND C., SØGAARD H., ELBRØND O., JØRGENSEN K. and ANDERSEN A.P.
(b): Epidermoid carcinoma of the tongue. Histologic grading in
the clinical evaluation. Acta Radiol. Ther. Phys. Biol.
14:(1975), 513.

LUND C., SØGAARD H., JØRGENSEN K. and HJELM-HANSEN M.:
Epidermoid carcinoma of the larynx. VI. Histologic grading in
the clinical evaluation. Acta Radiol. Ther. Phys. Biol.
15:(1976), 293.

NAHHAS W.A., SHARKEY F.E., WHITNEY C.W., HUSSEINZADEH N.,
CHUNG C.K. and MORTEL R.: The prognostic significance of
vascular channel involvement and deep stromal penetration in
early cervical carcinoma. Am. J. Clin. Oncol. (CCT) 6:(1983),
259.

VAN NAGELL J.R., DONALDSON E.S., PARKER, J.C., VAN DYKE A.H. and
WOOD E.G.: The prognostic significance of cell type and lesion
size in patients with cervical cancer treated by radical
surgery. Gynecol. Oncol. 5:(1977), 142.

VAN NAGELL J.R., DONALDSON E.S., WOOD E.G. and PARKER J.C.: The
significance of vascular invasion and lymphocytic infiltration
in invasive cervical cancer. Cancer 41:(1978), 228.

VAN NAGELL J.R., HUDSON, S., GAY E.C., DONALDSON E.S.,
HANSON M., POWELL D.F. and GOLDENBERG D.M.: Carcinoembryonic
antigen in carcinoma of the uterine cervix: Antigen distribution
in primary and metastatic tumours. Cancer 49:(1982), 379.

NØDSKOV-PEDERSEN S.: Degree of malignancy of cancer involving the cervix uteri, judged on the basis of clinical stage, histology, size of nuclei and content of DNA. Acta path. microbiol. scand. Sect. A 79:(1971), 617.

OSIRIS III. An integrated collection of computer programs for the management and analysis of social science data. Ann Arbor, Michigan, 1973.

POULSEN H.E., TAYLOR C.W. and SOBIN L.H.: Histological typing of female genital tract tumours. In: International histological classification of tumours. No. 13. WHO, Geneva 1975, 55.

RATTENBURY J. and. PELLETIER P.: Data processing in the social sciences with osiris. The University of Michigan, Ann Arbor, Michigan 48106, 1974.

REAGAN J.W. and FU Y.S.: Histologic types and prognosis of cancers of the uterine cervix. Int. J. Rad. Oncol. Biol. Phys. 5:(1979), 1015.

REAGAN J.W., HARMONIC M.J. and WENTZ W.B.: Analytical study of cells in cervical squamous cell carcinoma. Lab. Invest. 6:(1957), 241.

REAGAN J.W. and WENTZ W.B.: Genesis of carcinoma of uterine cervix. Clin. Obstet. Gynecol. 10:(1967), 883.

ROCHE W.D. and NORRIS H.J.: Microinvasive carcinoma of the cervix. The significance of lymphatic invasion and confluence patterns of stromal growth. Cancer 36:(1975), 180.

RUBIO C.A. and BIBERFELD P.: The basement membrane of the uterine cervix in dysplasia and squamous carcinoma: an immunofluorescent study with antibodies to basement membrane antigens. Acta path. microbiol. scand. Sect. A 83:(1975), 744.

- RUBIO C.A. and BIBERFELD P.: The basement membrane in experimentally induced atypias and carcinoma of the uterine cervix in mice. An immunofluorescence study. Virchows Arch. A. Path. Anat. and Histol. 381:(1979), 205.
- RUBIO C.A., BIBERFELD P. and EINHORN N.: The immunofluorescence characteristics of the basement membrane in squamous carcinoma of the uterine cervix. Histopathology 2:(1978), 67.
- RUITENBERG E.J., GUSTOWSKA L., ELGERSMA A. and RUITENBERG H.M.: Effect of fixation on the light microscopical visualisation of mast cells in the mucosa and connective tissue of the human duodenum. Int. Arch. Allergy 67:(1982), 233.
- SISSONS H.A.: Agreement and disagreement between pathologists in histologic diagnosis. Postgrad. med. J. 51:(1975), 685.
- SHINGLETON H.M., GORE H., SOONG S-J., ORR J.W., HATCH K.D., AUSTIN J.M. and PAIRIDGE E.E.: Tumor recurrence and survival in stage IB cancer of the cervix. Am. J. Clin. Oncol. 6:(1983), 265.
- STENDAHL U., EKLUND G., WILLÉN H. and WILLÉN R. (a): Invasive squamous cell carcinoma of the uterine cervix. III. A malignancy grading system for indication of prognosis after radiation therapy. Acta Radiol. Oncology 20:(1981), 231.
- STENDAHL U., EKLUND G. and WILLÉN R. (b): Invasive squamous cell carcinoma of the uterine cervix. IV. Analysis of a histopathologic malignancy grading system and construction of a partial index. Acta Radiol. Oncology 20:(1981), 289.
- STENDAHL U., EKLUND G. and WILLÉN R.: Prognosis of invasive squamous cell carcinoma of the uterine cervix: A comparative study of the predictive values of clinical staging IB-III and histopathologic malignancy grading system. Int. J. Gynecol. Pathol. 2(1983), 42.

STENDAHL U., LUNDBERG M. and WILLEÉN R.: Invasive squamous cell carcinoma of the uterine cervix. V. Late local recurrence after radiation therapy. Acta Radiol. Oncology 21:(1982), 81.

STENDAHL U., WILLEÉN H. and WILLEÉN R.: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. Acta Radiol. Oncology 18:(1979), 481.

STENDAHL U., WILLEÉN H. and WILLEÉN R.: Invasive squamous cell carcinoma of the uterine cervix. I. Definition of parameters in a histopathologic malignancy grading system. Acta Radiol. Oncology 19:(1980), 467.

STENDAHL U., WILLEÉN H. and WILLEÉN R. (c): Invasive squamous cell carcinoma of the uterine cervix. II. Reproducibility of a histopathologic malignancy grading system. Acta Radiol. Oncology 20:(1981), 65.

STENKVIST B., BENGTSSON E., ERIKSSON O., JARKRANS T. and NORDIN B. (a): A morphometric expression of differentiation in fine-needle biopsies of breast cancer. Cytometry 1:(1981), 292.

STENKVIST B., BENGTSSON E., ERIKSSON O., JARKRANS T. NORDIN, B. and WESTMAN-NAESER S. (b): Correlation between cytometric features and mitotic frequency in human breast carcinoma. Cytometry 1:(1981), 287.

STENKVIST B., BENGTSSON E., DAHLQVIST B., EKLUND G., ERIKSSON O., JARKRANS T. and NORDIN B.: Predicting breast prognosis. Cancer 50:(1982), 2884.

TATEISHI R., WADA A., HAYAKAWA K., HONGO J., ISHII S. and TERAOKAWA N.: Argyrophil cell carcinomas (Apudomas) of the uterine cervix. Light and electron microscopic observations of 5 cases. Virchows Arch. A Path. Anat. and Histol. 366:(1975), 257.

VALERI V., CRUZ, A.R., BRANDÃO H.J.S. and LISON L.: Relationship between cell nuclear volume and deoxyribonucleic acid of cells of normal epithelium of carcinoma in situ and of invasive carcinoma of the uterine cervix. Acta cytol. 11:(1967), 488.

te VELDE E.R., PERSIJN J.P., BALLIEUX R.E. and FABER J.: Carcinoembryonic antigen serum levels in patients with squamous cell carcinoma of the uterine cervix: clinical significance. Cancer 49:(1982), 1866.

WENTZ W.B.: Histogenesis of squamous cell carcinoma of the uterine cervix. In: New concepts of gynecological oncology, p. 51. Edited by G.C. Lewis, W.B. Wentz and R.M. Jaffe. Davies, Philadelphia 1966.

WENTZ W.B. and REAGAN J.W.: Survival in cervical cancer with respect to cell type. Cancer 12 (1959), 384.

WHITE F.H., MAYHEW T.M. and GOHARI K.: The application of morphometric methods to investigations of normal and pathological stratified squamous epithelium. Path. Res. Pract. 166:(1980), 323.

WILLÉN H. and EKLUND G.: Invasive squamous cell carcinoma of the uterine cervix. Construction of a partial index in a histopathologic malignancy grading system. Acta Radiol. Oncology (accepted for publication 1984).

WILLEN R., NATHANSON A., MOBERGER G. and ANNEROTH G.: Squamous cell carcinoma of the gingiva. Histological classification and grading of malignancy. Acta Otolarygol. 79:(1975) 146.

WILLÉN H., WILLEN R. and STENDAHL U.: Invasive squamous cell carcinoma of the uterine cervix. VI. Prediction value of non-keratinizing, parakeratotic and orthokeratotic cell forms and clinical staging. Acta Radiol. Oncology 21:(1982), 401.

WILLEN H., WILLÉN R. and STENDAHL U.: Invasive squamous cell carcinoma of the uterine cervix. VII. Influence of vehicle osmolarity on biopsy quality. Acta Radiol. Oncology 22:(1983), 257.

FROM THE DEPARTMENT OF ONCOLOGY, DIVISION OF GYNAECOLOGIC ONCOLOGY, AKADEMISKA SJUKHUSET S-750 14 UPPSALA, AND THE DEPARTMENT HISTOPATHOLOGY AND CYTOLOGY, FALU SJUKHUS, S-791 91 FALUN, SWEDEN.

INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

I. Definition of parameters in a histopathologic malignancy grading system

U. STENDAHL, H. WILLÉN and R. WILLÉN

Several attempts have been made to select treatment modality according to histopathologic evaluation of tumour biopsies. In 1893, VON HANSEMAN introduced the term anaplasia to denote an absence of differentiation. He believed that the degree of anaplasia was directly related to the invasive capacity of a tumour. This principle has been followed by most authors trying to evaluate the biologic activity of squamous cell carcinoma of the uterine cervix. However, the designs of the classification have been most variable:

SCHOTTLAENDER & KERMAUNER (1912) divided the tumours into 'reife, mittelreife und unreife' (mature, semi-mature and immature);

BRODERS (1920, 1925, 1926, 1940) and GOELLNER (1976) classified the tumours as of high, moderate and poor differentiation according to a scale from 1 to 4;

MARTZLOFF (1923, 1928) divided the tumours into spinal, transitional and fat-spindle cell types;

BÖHM & ZWEIFEL (1926) used four groups based on the Schottlaender & Kermauner classification, added keratinization and suggested the value of estimating the tumour-host relationship in terms of cellular response, eosinophilia and the amount of connective tissue;

HEALY & CUTTLER (1928) distinguished 3 grades (adult, plexiform and anaplastic) closely correspond-

ing to Martzloff's grades and Broders' grades 2 to 4.

HUEPER (1928 a, b) estimated both the tumour cell population and the tumour-host relationship by 20 different factors each grading from 1 to 4, the score constituting the malignancy index of tumour and correlating with survival. The parameters used were (1) special cell type of carcinoma, (2) nucleocytoplasmic coefficient, (3) number of 'pencil cells', (4) infiltrative cell growth, (5) general type of carcinoma, (6) irregular cell size, (7) irregular cell shape, (8) distinctness in outline of cells, (9) chromatism of cytoplasm, (10) functional activity of cells, (11) irregular size of nuclei, (12) irregular shape of nuclei, (13) chromatism of nuclei, (14) hyperchromatism of nuclei, (15) number of mitoses and prophase, (16) irregularity of mitoses, (17) character of stroma, (18) vascularity of stroma, (19) type of cellular infiltration of stroma and (20) amount of cellular infiltration of stroma.

WARREN (1931) and GATES & WARREN (1934) classified the carcinomas as low, medium and high malignant.

CHAMBERS (1935) grouped the tumours into adult, spindle and transitional types with the following subgroups: keratinized, differentiated, transitional and anaplastic. The latter divided into an alveolar

—

Submitted for publication 15 September 1980.

and a solid form. ACKERMAN & DEL REGATO (1947) used a histologic grading from 1 to 3 (high and moderate differentiation and an anaplastic type. TRUELSEN (1949) divided the squamous cell carcinomas into parakeratotic, squamous cell mucosal, undifferentiated and basal cell types.

GLUCKSMANN & SPEAR (1945), GILMOUR *et coll.* (1949) classified the tumours as anaplastic (A-tumours) and squamous cell differentiating tumours (D-tumours). In 1951, PENDL submitted his elaborate classification with 16 different subtypes:

A. Epidermoid carcinoma:

(1) Differentiated epidermoid carcinoma

- (I) Cutaneous type: (a) with horny lamellae, (b) predominantly with polygonal cells, (c) predominantly with spindle-shaped (basal) cells.

- (II) Mucosal type: (a) with all layers, (*) differentiated as usual, (**) with 'Holundermarkzellen', (b) one layer predominating, (*) predominantly spindle-shaped (basal) cells, (***) predominantly pale cells, (****) cuboid-columnar type of cell without gland formation. (*****) cuboid-columnar type of cell with gland formation.

- (2) Only slight differentiated epidermoid carcinoma, (a) small-celled intermediate type, (b) large-celled intermediate type.

B. Anepidermoid carcinoma:

- (1) Transitional-cell carcinoma,
- (2) Undifferentiated carcinoma.

C. Mixed type.

D. True adenocarcinoma.

E. Special types of carcinoma.

REAGAN *et coll.* (1957) and REAGAN & WENTZ (1967) classified the tumours into large cell non-keratinizing, large cell keratinizing and small cell carcinoma, a classification adopted by WHO (POULSEN *et coll.* 1975). GRAHAM & GRAHAM (1962) divided the tumours according to cytologic smears before and after radiation therapy into one group with good sensitization response (good SR) and the other with poor sensitization response (poor SR). A parabasal, a keratinizing, a pleomorphic and a small cell type grouping was used by TWEEDALE & RODDICK (1969).

Finally SEKIMOTO *et coll.* (1978a, b) designed a classification based on combination of maturation, functional differentiation and cell type. The latter was

divided into basal, squamous, prickle, pleomorphic, reserve and spindle cell type.

The intention of all these proposed classifications was to make it possible to predict the prognosis of the individual patient with fair certainty. However, the results varied and were often directly conflicting. Some authors found a good correlation to survival (for review see LANGE 1960), others denied that histologic grading had any prognostic value (LANGE, FIELD *et coll.* 1964, NG & ATKIN 1973, GOELLNER, JOHANSSON *et coll.* 1976). For the individual patient it was stated that no consistent guidelines as regards the future treatment of the patients could be based on the histologic appearances alone (JOHANSSON *et coll.*).

The light microscopic technique has been complemented with immunofluorescence and immunoperoxidase methods (BONFIGLIO & FEINBERG 1976, PERTSCHUK *et coll.* 1977, RUBIO *et coll.* 1978), and scanning electron microscopy (RUBIO & KRANTZ 1976, RUBIO & EINHORN 1977). Calculation of DNA content (ATKIN 1964, NØDSKOV-PEDERSEN 1971) and chromosome analyses (ATKIN & BAKER 1979) have been made. Computer analysis of exfoliated cells from the uterine cervix (HOLMQUIST *et coll.* 1978) and computer-aided cluster and discriminant analyses (KRAMER *et coll.* 1974) have also been attempted. These methods are still not easily accessible, but the aim has been to find objective and reproducible diagnostic criteria.

At the Department of Tumour Pathology of Radiumhemmet, Stockholm, a malignancy grading system, 8 parameters, each graded in 1 to 4 points, was developed to evaluate the biologic activities of squamous cell carcinoma of the larynx (JAKOBSSON 1973, 1975), palate (ENEROTH & MOBERGER 1973), gingiva (WILLÉN *et coll.* 1975). This system was used by LUND *et coll.* (1975a, b, 1976, 1977) in carcinomas of the lip, tongue and larynx, and by FISHER (1975) and HELWEG-LARSEN *et coll.* (1978) for the larynx. The present malignancy grading system was adopted and modified by STENDAHL *et coll.* (1979) for squamous cell carcinoma of the uterine cervix (8 parameters, each graded from 1 to 3 points, one new parameter introduced). In a retrospective investigation a high correlation was found between the sum of malignancy points and the clinical outcome of the individual. The present report gives a definition and a closer description of the parameters used.

Table 1

Parameters used for malignancy point grading. Tumour cell population

Parameter number		Points		
		1	2	3
1	Structure	Exophytic, papillary and solid	Small cords and groups of cells	Marked cellular dissociation
2	Differentiation into cell type	Large cell, no keratinization	Large cell keratinized	Small cell, no keratinization
3	Nuclear polymorphism	>75 per cent mature nuclei, few enlarged nuclei	75 to 25 per cent mature nuclei, moderate number of enlarged nuclei	<75 per cent mature nuclei, numerous irregular or anaplastic enlarged nuclei
4	Mitoses	Single 0-1	Moderate number 0-5	Numerous 0->5

Table 2

Parameters used for malignancy point grading. Tumour-host relationship

Parameter number		Points		
		1	2	3
5	Mode of invasion	Well defined borderline	Cords, less marked borderline	Groups of cells or diffuse growth
6	Stage of invasion	(Min. stroma inv.) or microcarcinoma	Nodular into submucosa and connective tissue	Massive; amongst muscles, and vessels
7	Vascular invasion	None	Possible	Well established within the lumina of lymph or blood vessels
8	Cellular response (plasmolymphocytic)	Marked (continuous rim)	Moderate (several large patches)	Slight or none (few small patches or no cells)

Methods

The tumour cell population and the tumour-host relationship were considered separately. The evaluation of the tumour cell population (Table 1) was based on the grading of cell differentiation, structure, nuclear polymorphism and the frequency of mitotic figures in terms of a 1 to 3 point scale. The tumour-host relationship (Table 2) was also estimated in terms of a 1 to 3 point scale by the mode of invasion, the stage of invasion, vascular invasion and the degree of lymphoplasmocytic infiltration. These 8 morphologic parameters permitted a grading with 8 to 24 points totally.

Comments

Tumour cell population

Structure (parameter 1) Magnification (4×12.5). It is important that the biopsy also includes stroma.

1 point. Papillary and solid types growing exophytically. Care should be taken that it is not an inverted growth with cords and groups of cells that penetrate the stroma (Fig. 1).

2 points. Small cords and groups of cells with retained cohesion. This group also includes aggregations of 15 to 20 cells sometimes combined with mesenchymal tissue (Fig. 2).

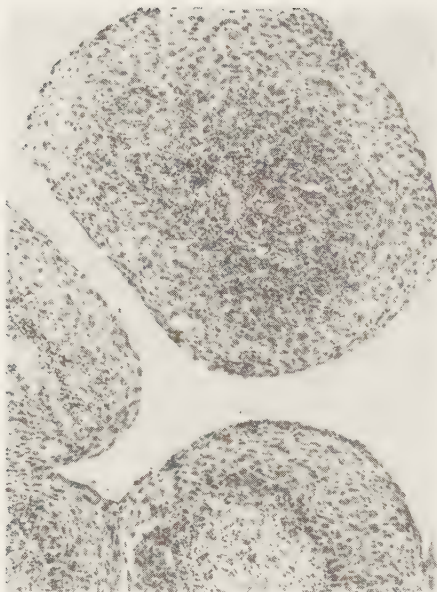


Fig. 1

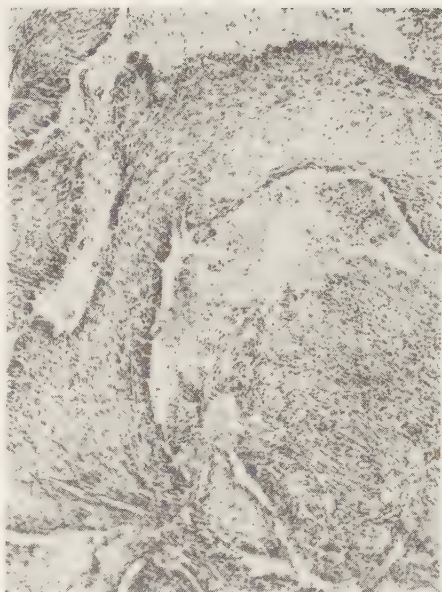


Fig. 2



Fig. 3

Fig. 1. Structure (parameter 1). Papillary type. One point.

Fig. 2. Structure (parameter 1). Small cords and groups of cells with retained cohesion. Two points.

Fig. 3. Structure (parameter 1). Marked cellular dissociation. Three points.

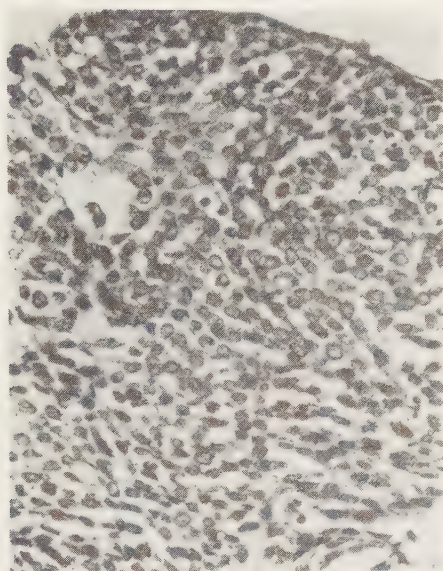


Fig. 4

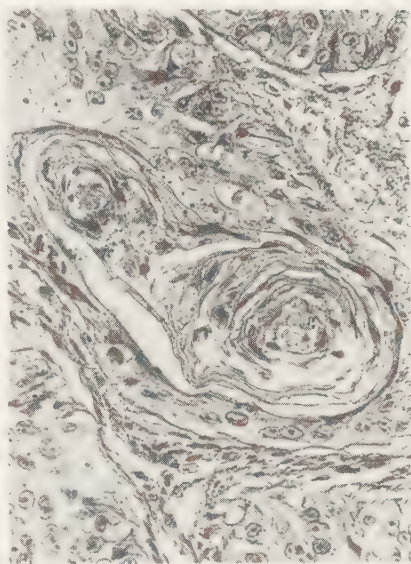


Fig. 5

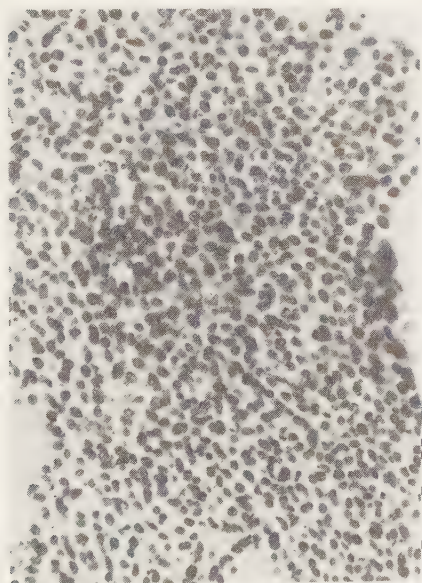


Fig. 6

Fig. 4. Differentiation into cell type (parameter 2). Large cell non-keratinizing type. One point.

Fig. 5. Differentiation into cell type (parameter 2). Large cell keratinizing type with pearl formation. Two points.

Fig. 6. Differentiation into cell type (parameter 2). Small cell non-keratinizing type with marked hyperchromatism. Three points.

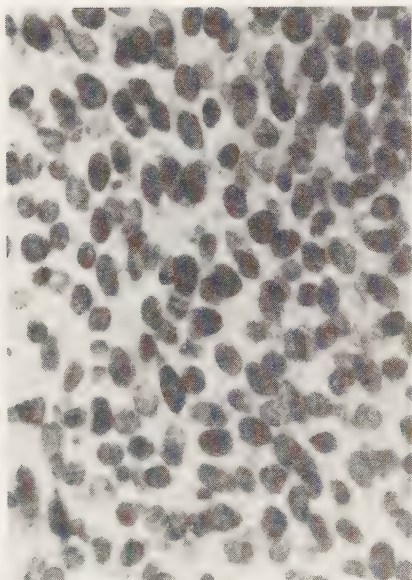


Fig. 7

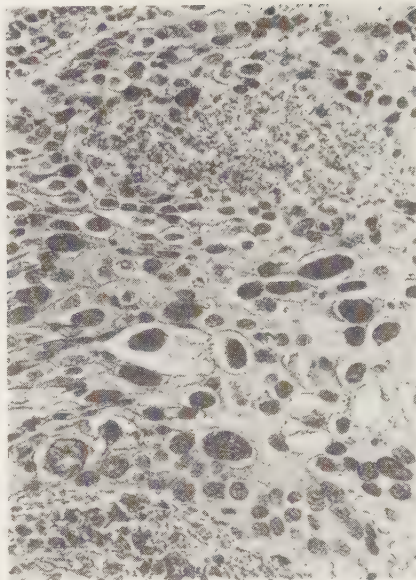


Fig. 8

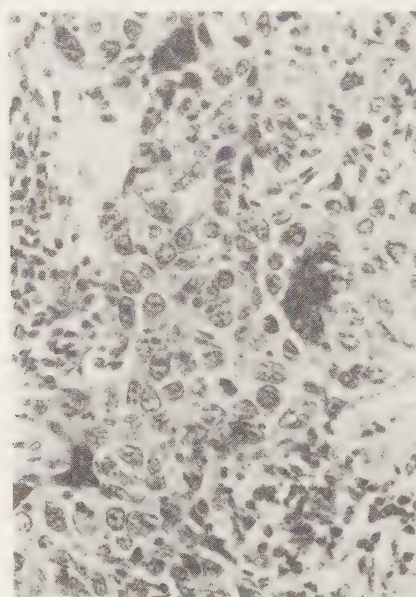


Fig. 9

Fig. 7. Nuclear polymorphism (parameter 3). Only slight nuclear variation with more than 75 per cent mature nuclei. One point.

Fig. 8. Nuclear polymorphism (parameter 3). Less than 75 per cent mature nuclei with solitary giant tumour cells and a moderate number of large nuclei. Two points.

Fig. 9. Nuclear polymorphism (parameter 3). Less than 25 per cent mature cells with numerous giant tumour cells, also bizarre containing tumour cell forms. Three points.

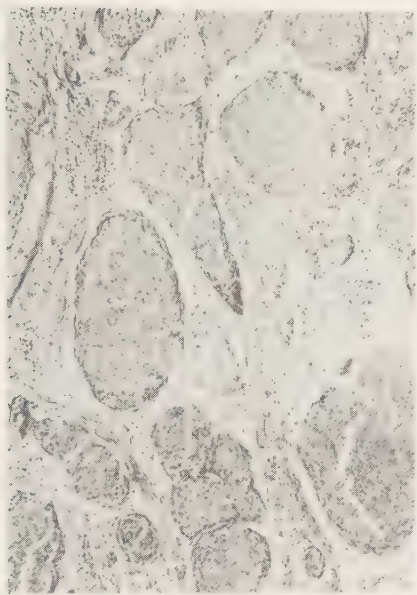


Fig. 10

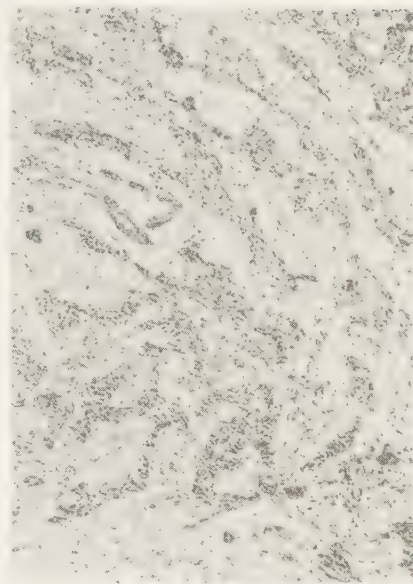


Fig. 11



Fig. 12

Fig. 10. Mode of invasion (parameter 5) Invasive squamous cell carcinoma with a defined borderline and clearly demarcated interface towards the stroma. One point.

Fig. 11. Mode of invasion (parameter 5). Less distinct borderline with disintegrating cords but the cells are still connected to each other. Two points.

Fig. 12. Mode of invasion (parameter 5). Strongly disintegrated appearances with individual cells or small cell groups. Three points.

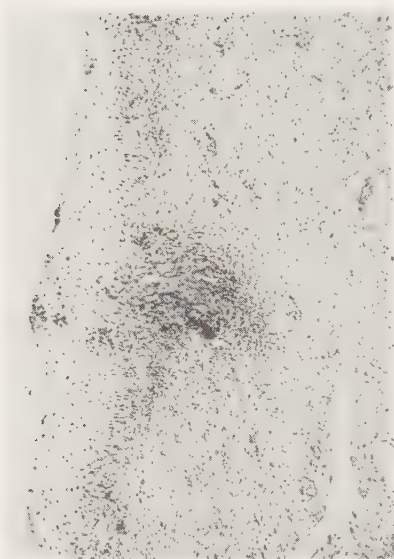


Fig. 13

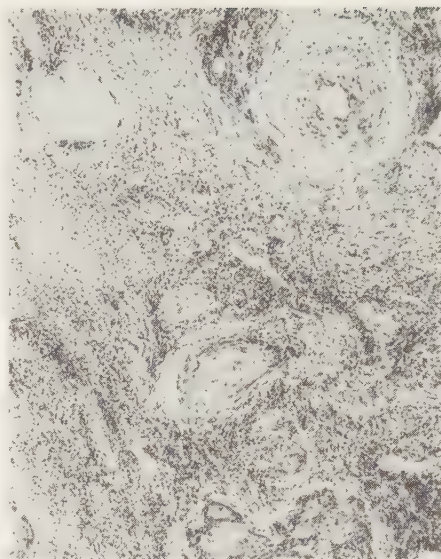


Fig. 14

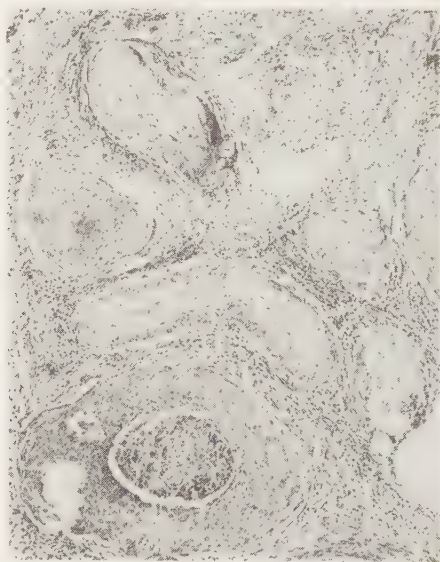


Fig. 15

Fig. 13. Stage of invasion (parameter 6). Slight invasion into stroma. One point.

Fig. 14. Stage of invasion (parameter 6). Invasive squamous cell carcinoma with growth into stroma and collagen tissue, not reaching the medium sized vessels or the muscles. Two points.

Fig. 15. Stage of invasion (parameter 6). Growth close to and between medium sized vessels and also growth into the musculature. Three points.

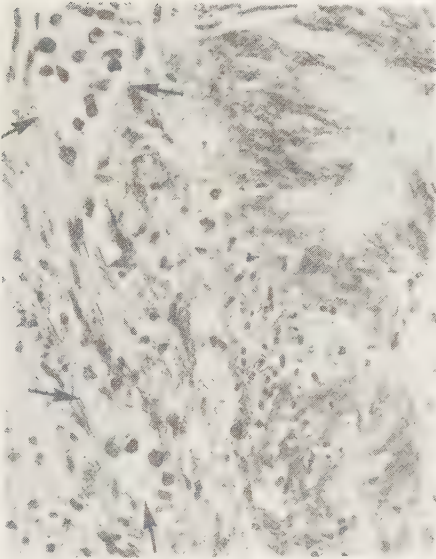


Fig. 16. Vascular invasion (parameter 7). Invasive squamous cell carcinoma within the vessel wall (→) but no evidence of intra-luminal manifestations. Two points.

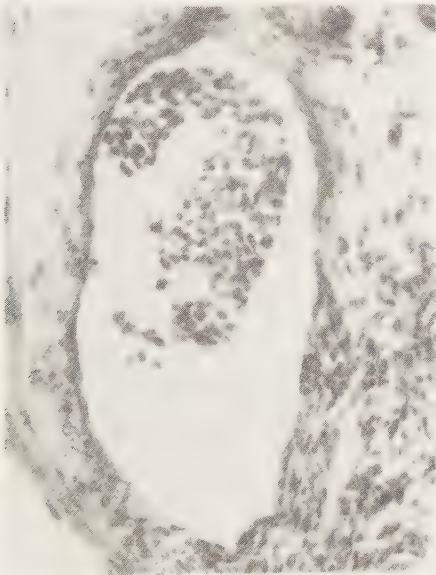


Fig. 17. Vascular invasion (parameter 7). Well established growth of tumour cells within the lumen of a blood vessel. Three points.

3 points. Marked cellular dissociation. Only a few cells may cling together (Fig. 3).

Differentiation into cell type (parameter 2) follows the definition given by WHO (POULSEN et coll.). Generally only one cell type is found but in mixtures of several kinds, for example, large aggregations of basaloid cells in combination with large cell formations, points are given to the lowest differentiated form (STENDAHL et coll.).

1 point. Large cell, non-keratinizing type. This is the most frequent sub-type. Keratinization is absent or limited to isolated cells. Sometimes these tumours are composed of cells with abundant clear cytoplasm (Fig. 4).

2 points. Large cell keratinizing type. This sub-type is characterized by tumour cells with pearl-formation. Strongly keratinized parakeratotic forms are also included in this group even if only small amounts of keratine are found in intermediate and basal cell layers. One to 3 cell layers with negligible keratinization in the surface area are classified with 1 point (Fig. 5).

3 points. Small cell, non-keratinizing type. This type is rare (2 to 10 per cent). No keratinization is found. The cells are smaller but the relative nuclear size is increased. Hyperchromatism of the nuclei is marked (Fig. 6).

Nuclear polymorphism (parameter 3) This is a modification of Broders' system (BRODERS 1920, 1925, 1926, 1940). Thirty high power fields are examined (40×12.5).

1 point. Requires more than 75 per cent mature nuclei and only negligible size variations (Fig. 7).

2 points. Requires 25 to 75 per cent mature nuclei, solitary giant tumour cells and a moderate number of enlarged nuclei (Fig. 8).

3 points. Requires less than 25 per cent mature cells, extremely irregular, often numerous giant tumour cells and bizarre tumour cell forms. Extremely immature cells of basaloid type are included (Fig. 9).

Mitoses (parameter 4). Magnification (40×12.5). This parameter requires very accurate classification. Thirty high-power fields are examined. The counting must not only be related to the basal cell layer but also to the prickle-cell and superficial layers. Clear chromosome pattern should be distinguishable to avoid interpreting hyperchromatic cell nuclei or pycnotic cell nuclei as mitoses.

1 point. 0 or 1 mitosis per high-power field.

2 points. 2 to 5 mitoses per high-power field.

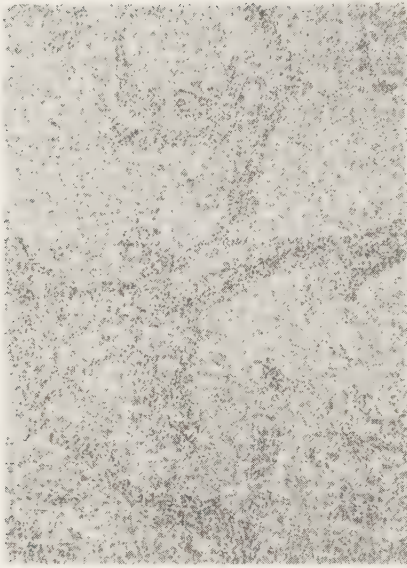


Fig. 18. Lymphoplasmacytic cell response (parameter 8). Marked cellular reaction around and within the tumour nodules. One point.

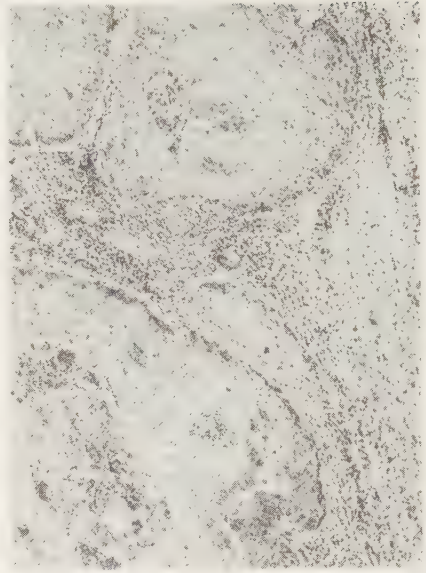


Fig. 19. Lymphoplasmacytic cell response (parameter 8). Unevenly distributed aggregates of lymphoplasmacytic cells, in some areas nearly no lymphocytes. Two points.

3 points. More than 5 mitoses per high-power field.

Tumour-host relationship

Mode of invasion (parameter 5). Magnification (10×12.5). Attention should be paid to fixation artefacts, mechanical fragmentation and some disintegration because of an inflammatory reaction in connection with intensive lympho-plasmacytic or lichenoid reaction.

1 point. Defined border-line with a pushing appearance where the interface continues to be clearly demarcated. When border-line is slightly less distinct on account of an inflammatory reaction covering some cell layers the tumour is included in this group (Fig. 10).

2 points. Less distinct border-line. The carcinoma penetrates the stroma with pointed disintegrating cords, still connected to each other (Fig. 11).

3 points. Strongly disintegrated appearance. Individual cells or groups up to 10 cells. Round aggregates containing 10 to 15 cells or more, often with a marked hyaline membrane, are given 1 point (Fig. 12).

Stage of invasion (parameter 6). The biopsy should include all cell layers also including the lamina muscularis.

1 point. Minimum stromal invasion or a microcarcinoma is defined according to WHO (POULSEN et coll.): 'Minimal stromal invasion is carcinoma in situ with areas giving suspicion of penetration into the underlying stroma. Microcarcinoma is defined as stromal invasion limited to isolated microscopic foci' (generally 5 mm or less in depths) (Fig. 13).

2 points. Growth into stroma and collagen tissue where the tumour should not penetrate deeper than one vessel diameter from vessels of medium size. The border-line between 2 and 3 points may sometimes be difficult to detect. A deep biopsy and accurate examination of tumour growth in relation to the medium-sized vessels is necessary (Fig. 14).

3 points. Growth close to medium-sized vessels or between the vessels, and growth into the musculature (Fig. 15).

Vascular invasion (parameter 7). This parameter is difficult to estimate. Fixation artefacts and mechanical fragmentation that easily give spaces with intima-like covering must be identified.

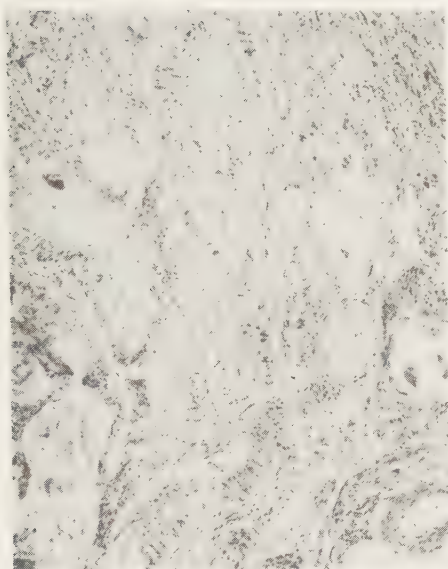


Fig. 20. Lymphoplasmocytic cell response (parameter 8). Slight cell reaction in the stroma. No reaction within the tumour nodules. Three points.

1 point. No vascular invasion.

2 points. Growth around vessels with compressive effect or infiltrating the wall without reaching the lumen (Fig. 16).

3 points. Clear and well-established growth of tumour cells into the lumen of lymphatics or blood vessels (Fig. 17).

Lymphoplasmocytic cell response (parameter 8). Magnification (10×12.5).

1 point. Marked cellular reaction with a continuous border of lymphoplasmocytic cells around the tumour nodules in many places (Fig. 18).

2 points. Unevenly distributed aggregates of lymphoplasmocytic cells, other areas sparsely covered with lymphocytes (Fig. 19).

3 points. Minimum or no lymphoplasmocytic cell reaction, e.g. solitary, small clusters, minimum amount of dissociated cells, or no lymphoplasmocytic cells at all (Fig. 20).

Discussion

Microscopic classification of squamous cell carcinoma was presented by VON HANSEMAN, SCHOTTLAENDER & KERMAUNER and BRODERS

(1920, 1925, 1926, 1940) mainly as a monofactorial system based on cellular differentiation. A number of similar systems have later followed (for review see LANGE).

It was early realized that the connective tissue had a capacity to prevent progression of the tumour and the tumour-host relationship was carefully analysed: SCHOTTLAENDER & KERMAUNER described different tumour-connective tissue relations: medullary tumours in which the connective tissue made up a small part, a scirrhous type with preponderance of connective tissue, and finally mixed types. Scirrhous tumours are rather uncommon and were considered to offer the best prognosis. ADLER (1916) stated that apart from the type of connective tissue, the vascularity of the tumour might influence the prognosis. He gave 4 possible combinations of which tumours rich in connective tissue and sparsely vascularized were supposed to have the best prognoses following radiation therapy.

LAHM (1927) divided the connective-tissue reaction around the tumour into 4 grades. Grade 1: hyperaemia and round-cell infiltration, grade 2: granulation tissue in the interface between tumour-parenchyma and the underlying connective tissue, grade 3: no cellular reaction, and grade 4: sclerotic connective tissue. Prognosis was said to be best in grade 4. LAX (1950) classified the connective-tissue reaction into 3 groups: (1) satisfactory new-formation of connective tissue with regular arrangement and ample fibres, (2) poor reaction with no or little formation of fibres, and (3) shrinkage, hyalinization, and partial breakdown of the connective tissue with rupture and splitting of the fibres. In this system prognosis deteriorated from 1 to 3. LINELL & MÄNSSON (1952) evaluated the amount and type of stroma but found no relation to survival. PAAVOLAINEN (1970) and PAAVOLAINEN *et al.* (1973) found an increased frequency of local recurrences when acid mucopolysaccharides increased in the stroma.

Contrary to LAHM, SCHOCK (1926) and STUPER (1953 a, b), LINELL & MÄNSSON could not find that a large number of eosinophilic cells was correlated to better prognosis.

An increased number of granulocytes resulted in decreased survival (HUEPER & SCHMITZ 1927, HUEPER 1928 a, b). Tumour necrosis was also correlated to higher mortality (KOTTMEIER 1955, GLUCKSMANN & SPEAR). An enhancement of lymphocytes into the stroma was said to reduce the

frequency of metastasis (CHERRY & GLÜCKSMANN 1955, GUSBERG *et coll.* 1971).

BOHM & ZWEIFEL, LINELL & MÄNSSON as well as CULLHED (1978) reported that absence of lymphocytic infiltration was an unfavourable prognostic sign of survival. However, BARBER *et coll.* (1978) found no such correlation. CULLHED likewise demonstrated a significant low frequency of lymph gland metastasis when a high rate of lymphocytes around the tumour complexes was encountered. The growth of the carcinoma into the underlying stroma was observed with attention paid to the mode of infiltration. Tumours with ribbon and bandlike configurations had a better clinical outcome than those with complete cellular dissociation (SCHOTTLAENDER & KERMAUNER, STÜPER 1953 a, b, LAX, BEECHAM *et coll.* 1978, CULLHED).

The importance of vascular invasion is evident and patients with such a feature face worse clinical outcome in both operated and irradiated patients (STEIN-WERBLOWSKY 1955, CHERRY & GLÜCKSMANN, BARBER *et coll.* CULLHED).

Several multifactorial classification systems have been developed. HUEPER (1928 a, b) devised an index of malignancy by which the sensitivity of the tumour to radiation could be assessed. It was claimed to correlate well with survival. The complicated classification, giving points for 20 different factors, made the system highly subjective and has not become widely used. PENDL's classification is very elaborate but requires a large material of patients. Groups 2, 3, 6, and 13 comprise 60 to 65 per cent of all patients (PENDL, SCHÜLLER 1952, STÜPER 1953 a, b) and the other 11 groups constitute the remaining 35 to 40 per cent. In STÜPER's large series of 815 patients, 9 of the groups comprised less than 40 patients and 4 less than 10 patients. However, well-founded the PENDL classification may be, morphologically and theoretically it does not appear to be suitable for routine use.

SCHÜLLER demonstrated that PENDL's classification could also be used when reaching decision whether a patient should be operated upon or irradiated. CULLHED used several of the parameters used in the present material but made no attempt to calculate total malignancy points. The functional classification of SEKIMOTO *et coll.* (1978 a, b) has been published in short communications only. The clinical value remains to be proven.

The present malignancy grading system includes a description of the tumour cell population and the

tumour-host relationship and is an attempt to create a simple and reproducible method. The parameters were chosen on the basis of the good results previously obtained by ENEROTH & MOBERGER, WILLÉN *et coll.*, LUND *et coll.* (1975 a, b, 1976, 1977), CULLHED and STENDAHL *et coll.* but these parameters will not necessarily be those finally used. The prognostic value of the parameters may also be revised. A multifactorial analysis is at present in progress which will provide further information in this respect. Preliminary results indicate that most of the prognostic information is found in the tumour-host relationship.

It is essential that all parameters can be identified in the biopsy and that characteristics of the invasive zone are not underestimated. This is in agreement with JAKOBSSON (1973) but is contrary to the results of LUND *et coll.* (1975 a, b, 1976, 1977) and HELWEG-LARSEN *et coll.*, thus implying that large and deep biopsies must be taken in order to fit into the grading system. The biopsy should also be large in order to reduce the sampling error as parameters such as cell type, nuclear polymorphism, mitoses and mode of invasion may be unevenly distributed in the tissue (MARTZLOFF 1928, SILVERBERG 1976, CHI *et coll.* 1977).

SUMMARY

A histologic malignancy grading system for invasive squamous cell carcinoma of the uterine cervix is presented. The method is based upon evaluation of the tumour cell population in terms of cell differentiation, structure, nuclear polymorphism and the frequency of mitotic figures in terms of a 1 to 3 point scale. The tumour-host relationship was also estimated in terms of a 1 to 3 point scale, by the mode of invasion, stage of invasion, vascular invasion and degree of lymphoplasmocytic infiltration. These parameters permitted a grading with 8 to 24 points totally.

ACKNOWLEDGEMENTS

Financial support was received from Stockholms Cancerförening. The authors wish to thank Bengt Holmquist and Nils Lindgren for expert library service and Gösta Andersson for photography.

REFERENCES

- ACKERMAN L. V. and DEL REGATO J. A.: *Cancer. Diagnosis, treatment and prognosis*. First edition. C. V. Mosby, St. Louis 1947.

- ADLER L.: Morphologische Kennzeichen für die Radiumempfindlichkeit der Karzinome des weiblichen Genitales. *Zbl. Gynäk.* 40 (1916), 673.
- ATKIN N. B.: Nuclear size in carcinoma of the cervix. Its relation to DNA content and to prognosis. *Cancer* 17 (1964), 1391.
- and BAKER M. C.: Chromosome 1 in 26 carcinomas of the cervix uteri. Structural and numerical changes. *Cancer* 44 (1979), 604.
- BARBER H. R. K., SOMMERS S. C., RATTERDAM H. and KWON T.: Vascular invasion as a prognostic factor in stage I B cancer of the cervix. *Obstet and Gynec.* 52 (1978), 343.
- BEECHAM J. B., HALVORSEN T. and KOLBENSTVEDT A.: Histologic classification, lymph node metastases, and patient survival in stage IB cervical carcinoma. An analysis of 245 uniformly treated cases. *Gynec. Oncol.* 6 (1978), 95.
- BOHM D. and ZWEIFEL E.: Inwieweit kann man heute aus mikroskopischen Befunden eine Prognose für die Bestrahlung des Uteruskarzinoms stellen? *Zbl. Gynäk. Geburtsh.* 1 (1926), 30.
- BONFIGLIO T. A. and FEINBERG M. R.: Isoantigen loss in cervical neoplasia. Demonstration by immunofluorescence and immuno-peroxidase techniques. *Arch. Path. Lab. Med.* 100 (1976), 307.
- BRODERS A. C.: Squamous cell epithelioma of the lip. *J. Amer. med. Ass.* 74 (1920), 656.
- The grading of carcinoma. *Minn. Med.* 8 (1925), 726.
- Carcinoma grading and practical application. *Arch. Path. (Chic.)* 2 (1926), 376.
- Microscopic grading of cancer. In: *Treatment of cancer and allied diseases*, p. 9. Edited by G. T. Pack and E. M. Livingstone. Paul B. Hoeber, New York 1940.
- CHAMBERS H.: The histological classification of cancers of the uterine cervix and the relation between the growth structure and the results of radium treatment. *Amer. J. Cancer* 23 (1935), 1.
- CHERRY C. P. and GLUCKSMANN A.: Lymphatic embolism and lymph-node metastasis in cancers of vulva and of uterine cervix. *Cancer* 8 (1955), 564.
- CHI C. H., RUBIO C. A. and LAGERLÖF B.: The frequency and distribution of mitotic figures in dysplasia and carcinoma in situ. *Cancer* 39 (1977), 1218.
- CULLHED S.: Carcinoma cervicis uteri Stages I and IIa. Treatment-histopathology-prognosis. Thesis. Linköping 1978.
- ENEROTH C. M. and MÖBERGER G.: Histological malignancy grading of squamous cell carcinoma of the palate. *Acta oto-laryng. (Stockh.)* 75 (1973), 293.
- FIELD D. A., DOCKERTY M. B. and SYMONDS R. E.: Small cell cancer of the cervix. *Amer. J. Obstet. Gynec.* 88 (1964), 447.
- FISHER H. R.: Grading of biopsies of laryngeal carcinomas by multiple criteria. *Canad. J. Otolaryng.* 4 (1975), 881.
- GATES O. and WARREN S.: The grading of epidermoid carcinoma. *Surg. Gynec. Obstet.* 58 (1934), 962.
- GILMOUR M., GLUCKSMANN A. and SPEAR F. G.: The influence of tumour histology, duration of symptoms and age of patients on the radiocurability of cervix tumours. *Brit. J. Radiol.* 22 (1949), 90.
- GLUCKSMANN A. and SPEAR F. G.: The qualitative and quantitative histological examination of biopsy material from patients treated by radiation for carcinoma of the cervix uteri. *Brit. J. Radiol.* 18 (1945), 313.
- GOELLNER J. R.: Carcinoma of the cervix. Clinicopathologic correlation of 196 cases. *Amer. J. clin. Path.* 66 (1976), 775.
- GRAHAM R. M. and GRAHAM J. B.: Cytologic prognosis in cancer of cervix. Two year survival rates in randomized series. *Amer. J. Roentgenol.* 87 (1962), 56.
- GUSBERG S. B., YANNOPoulos K. and COHEN C. J.: Virulence indices and lymph nodes in cancer of the cervix. *Amer. J. Roentgenol.* 111 (1971), 273.
- VON HANSEMAN D.: Studien über die Spezifität den Altruismus und die Anaplasia der Zellen. A. Hirschwald, Berlin 1893.
- HEALY W. and CUTTLER M.: Relation between structure and prognosis in cervical carcinoma under radiation treatment. *Amer. J. Obstet.* 16 (1928), 15.
- HELWEG-LARSEN K., GRAEM N., MEISTRUP-LARSEN K. I. and MEISTRUP-LARSEN U.: Clinical relevance of histological grading of cancer of the larynx. *Acta path. microbiol. scand. Sect. A.* 86 (1978), 499.
- HUEPER W. C. (a): The relation of the histological structure to the prognosis of the carcinomas of the uterine cervix. *Surg. Gynec. Obstet.* 47 (1928), 502.
- (b): Carcinomas of the uterine cervix. Their histologic structure, malignancy and prognosis. *Arch. Path.* 6 (1928), 1064.
- und SCHMITZ H.: Der histologische Malignitätsindex und seine Bedeutung für Prognose und Behandlung der Cervixcarcinome des Uterus. *Strahlentherapie* 24 (1927), 660.
- — Der prognostische Wert des 'histologischen Malignitätsindex' und der 'klinischen Einleitung' der Cervixcarcinome der Uterus. *Strahlentherapie* 30 (1928), 650.
- HOLMQUIST J., BENGTSSON E., ERIKSSON O., NORDIN B. and STENKVIST B.: Computer analysis of cervical cells. Automatic feature extraction and classification. *J. Histochem. Cytochem.* 26 (1978), 1000.
- JAKOBSSON P. Å.: Glottic carcinoma of the larynx. Factors influencing prognosis following radiotherapy. Thesis. Karolinska Institutet, Stockholm 1973.
- Histologic grading of malignancy and prognosis in glottic carcinoma of the larynx. *Canad. J. Otolaryng.* 4 (1975), 885.
- JOHANSSON O., JOHANSSON J. E., LINDBERG L. G. and SYDSJÖ A.: Prognosis, recurrences and metastases correlated to histologic cell type in carcinoma of the uterine cervix. *Acta obstet. gynec. scand.* 55 (1976), 255.
- KOTTMEIER H. L.: The place of radiation therapy and of surgery in the treatment of uterine cancer. *J. Obstet. Gynaec. Brit. Emp.* 62 (1955), 737.
- KRAMER I. R. H., EL-LABBAN N. G. and SONKODI S.: Further studies on lesions of the oral mucosa using computer-aided analyses of histological features. *Brit. J. Cancer* 29 (1974), 223.

- LAHM W.: Die Bedeutung der mikroskopischen Untersuchung für die Behandlung und Prognose des Collumkarzinoms. *Ergebn. Strahlenforsch.* 1 (1927), 556.
- LANGE P.: Clinical and histopathological studies on cervical carcinoma. *Acta path. microbiol. scand.* 50 (1960) Suppl. No. 143.
- LAX H.: Die Prognose der Kollumkarzinome auf Grund histologischer Beurteilung. *Zbl. Gynäk.* 72 (1950), 284.
- LINELL F. and MÅNSSON B.: Prognostic value of histologic grading of carcinoma of cervix uteri. A study of 388 cases treated with radium and roentgen therapy. *Acta radiol.* 38 (1952), 219.
- LUND C., SØGAARD H., JØRGENSEN K. and HJELM-HANSEN M.: Epidermoid carcinoma of the larynx. IV. Histologic grading in the clinical evaluation. *Acta radiol. Ther. Phys. Biol.* 15 (1976), 293.
- ELBROND O., JØRGENSEN K. and ANDERSEN A. P. (a): Epidermoid carcinoma of the lip. Histologic grading in the clinical evaluation. *Acta radiol. Ther. Phys. Biol.* 14 (1975), 465.
- — — — (b): Epidermoid carcinoma of the tongue. Histologic grading in the clinical evaluation. *Acta radiol. Ther. Phys. Biol.* 14 (1975), 513.
- JØRGENSEN K., ELBROND O., HJELM-HANSEN M. and ANDERSEN A. P.: Histologic grading of epidermoid carcinomas in the head and neck. *Danish med. Bull.* 24 (1977), 162.
- MARTZLOFF K. H.: Carcinoma of cervix uteri. Pathological and clinical study with particular reference to relative malignancy of neoplastic process as indicated by predominant type of cancer cell. *Bull. Johns Hopk. Hosp.* 34 (1923), 141.
- Epidermoid carcinoma of cervix uteri. Histologic study to determine resemblance between biopsy specimens and parent tumour obtained by radical panhysterectomy. *Amer. J. Obstet. Gynec.* 16 (1928), 578.
- NG A. B. P. and ATKIN N. B.: Histological cell type and DNA value in the prognosis of squamous cell cancer of uterine cervix. *Brit. J. Cancer* 28 (1973), 322.
- NØDSKOV-PEDERSEN S.: Degree of malignancy of cancer involving the cervix uteri judged on the basis of clinical stage, histology, size of nuclei and content of DNA. *Acta path. microbiol. scand. Sect. A* 79 (1971), 617.
- PAAVOLAINEN M.: Stromal reactions as prognostic factors in epidermoid carcinoma of the larynx. Thesis, Helsinki 1970.
- TARKKANEN J. and SAKSELA E.: Stromal reactions as prognostic factors in epidermoid carcinoma of the tongue. *Acta oto-laryng. (Stockh.)* 75 (1973), 316.
- PENDL O.: Histologische Klassifizierung und Ergebnisse der Strahlenbehandlung des Carcinoma colli uteri. *Radiol. austriaca* 4 (1951), 95.
- PERTSCHUK L. P., BOYCE J. G. and URCUYO R.: An immunofluorescent study of basement membranes in squamous cell carcinoma of the cervix, vagina and vulva. *Obstet. and Gynec.* 49 (1977), 417.
- POULSEN H. E., TAYLOR C. W. and SOBIN L. H.: Histological typing of female genital tract tumours. In: International histological classification of tumours. No. 13. WHO, Geneva 1975.
- REAGAN J. W. and WENTZ W. B.: Genesis of carcinoma of uterine cervix. *Clin. Obstet. Gynec.* 10 (1967), 883.
- HARMONIC M. J. and WENTZ W. B.: Analytical study of cells in cervical squamous cell cancer. *Lab. Invest.* 6 (1957), 241.
- RUBIO C. A. and EINHORN N.: The exfoliating epithelial surface of the uterine cervix. IV. Scanning electron microscopical study in invasive squamous carcinoma of human subjects. *Beitr. Path.* 161 (1977), 72.
- and KRANZ I.: The exfoliating cervical epithelial surface in dysplasia, carcinoma in situ and invasive squamous carcinoma. I. Scanning electron microscopic study. *Acta cytol.* 20 (1976), 144.
- BIBERFELD P. and EINHORN N.: The immunofluorescence characteristics of the basement membrane in squamous carcinoma of the uterine cervix. *Histopathology* 2 (1978), 67.
- SCHOCH E. U.: Über die lokale Eosinophilie bei Karzinom. *Zbl. Gynäk.* 45 (1926), 2895.
- SCHOTTLAENDER J. and KERMAUNER F.: Zur Kenntnis des Uterus-Karzinoms. S. Karger, Berlin 1912.
- SCHÜLLER E.: Beziehungen des histologischen Tumortypus des Collumcarcinoms zur Dauerheilung durch Operation und seine Bedeutung für Prognose und Therapie dieser Erkrankung. *Arch. Gynäk.* 181 (1952), 360.
- SEKIMOTO K., NAKANO M. and SATOH S. (a): Sequential cytopathological changes of epidermoid carcinoma of the uterine cervix. I. Comparison between type base and type sqm. *J. Jap. Cancer Clin.* 24 (1978), 203.
- TANAKA N. and KENO T. (b): Sequential cytopathological changes following irradiation of epidermoid carcinoma of the uterine cervix of spindle cell type in our classification. *J. Jap. Cancer Clin.* 24 (1978), 603.
- SILVERBERG S. G.: Reproducibility of the mitosis count in the histologic diagnosis of smooth muscle tumors of the uterus. *Human Path.* 7 (1976), 451.
- STENWERBLOWSKY R.: Prognosis in cancer of the cervix uteri. *Brit. J. Radiol.* 28 (1955), 623.
- STENDAHL U., WILLEN H. and WILLEN R.: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. *Acta. radiol. Oncology* 18 (1979), 481.
- STUPER P. (a): Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome. I. Mitteilung. Untersuchungen über die histologische Klassifizierung nach Pendl. *Strahlentherapie* 92 (1953), 89.
- (b): Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome. 4. Mitteilung. Untersuchungen zur Frage der 'elektiven Therapie'. *Strahlentherapie* 92 (1953), 338.
- TRUELSEN F.: Cancer of the uterine cervix. Rosenkilde and Bagger, Copenhagen 1949.
- TWEEDALE D. N. and RODDICK JR J. W.: Histologic types of squamous cell carcinoma in situ of the cervix. *Obstet. and Gynec.* 33 (1969), 35.
- WARREN S.: Grading of carcinoma of the cervix as checked at autopsy. *Arch. Path. (Chic.)* 12 (1931), 783.
- WILLEN R., NATHANSSON A., MOBERGER G. and ANNEROTH G.: Squamous cell carcinoma of the gingiva. Histological classification and grading of malignancy. *Acta oto-laryng. (Stockh.)* 79 (1975), 146.

INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

VII. Influence of vehicle osmolarity on biopsy quality

H. WILLÉN, R. WILLÉN and U. STENDAHL

The introduction of a histopathologic malignancy grading system for prediction of the prognosis in individual patients with squamous cell carcinoma of the uterine cervix has made it necessary to pay special attention to biopsy sampling, fixation and staining techniques. Vascular invasion has proved to be a valuable prognostic parameter, the estimation of which is important but often uncertain because of shrinkage or fragmentation of the biopsy specimen. In order to separate quantitative as well as qualitative changes more adequately, to improve grading, better fixation methods were required.

Evidence has accumulated showing that the *total* osmotic pressure of the fixative has less importance than that of the fixative vehicle (BRUNK et coll. 1975, COLLINS et coll. 1977) and that formaldehyde exerts little effective osmotic pressure in buffer systems (ARBORGH et coll. 1976, COLLINS et coll.). The *effective* osmotic pressure is mainly exerted by the fixative vehicle (HAYAT 1970, BOYDE & VESELY 1972). This pressure is produced by those molecules and ions which do not freely pass the semipermeable membrane (HOPWOOD 1969). The effective osmotic pressure should be separated from the total osmotic pressure of the fixative measured by freeze point depression (ARBORGH et coll.). Thus the optimal osmotic pressure of the fixative is mainly dependent on the properties of the vehicle (BRUNK et coll.).

Several analyses of vehicle osmolarity and the importance of aldehydes on fixation properties have

been performed for electron microscopy (BRUNK et coll., ARBORGH et coll., COLLINS et coll., HOPWOOD 1969, 1972). In contrast, very few investigations have been made concerning fixation for conventional light microscopic examination (BEYER-BOON et coll. 1979, DAVENPORT & BALL 1979, RUITENBERG et coll. 1982). In the present analysis, the optimal vehicle osmolarity has been examined for both carcinoma *in situ* and invasive squamous cell carcinoma of the uterine cervix. The cervical tissue consists of firm fibrous components such as muscular tissue, vessels and nerves in combination with epithelial cells with varying cohesion to the underlying stroma. Artefacts may occur because of suboptimal fixation and mechanical trauma. A standard technique for sampling, fixation and staining of the specimen is most important when malignancy grading is performed on surgically excised carcinomas (STENDAHL et coll. 1979, 1980, 1981).

A pair of biopsy forceps has been designed in order to obtain a representative specimen including tumour and the underlying stroma with as little damage to the specimen as possible. The aim of the present investigation was to define the optimal effective osmolarity for the fixing of clinical biopsies from preinvasive and invasive squamous cell carcinomas of the uterine cervix.

From the Departments of Histopathology and Oncology, Gynecologic Section, University Hospital, S-221 85 Lund, Sweden. Accepted for publication 7 January 1983.

Material and Methods

The material consisted of 15 cones from patients with carcinoma *in situ* and of biopsies taken from 45 patients with invasive squamous cell carcinoma, stages I to IV. The cone material from each patient was cut into 8 fractions and put into the following solutions for 24 hours: (1) 10% unbuffered formalin (1:10 diluted commercial formaldehyde) given a total osmolarity <1600 mmol/l and an effective osmolarity of <50 mmol/l, (2) 10% formalin in phosphate buffer, pH 7.0, with a molarity of 0.05 (100), 0.09 (200), 0.12 (250), 0.15 (310), 0.17 (350), 0.20 (400) and 0.26 (510), respectively. Effective osmolarity within brackets (mmol/l).

Two biopsies were taken from each patient with invasive squamous cell carcinoma. One was fixed in either 0.15 (310), 0.17 (350) or 0.26 (510) molar phosphate buffer, pH 7.0, with 10% formalin and the other in conventional 10% unbuffered formalin (<50). The material was then processed in a routine fashion, embedded in paraffin, 4 to 5 μ m slides prepared and stained with hematoxylin-eosin, van Gieson stain for fibrous tissue, PAS for glycogen and neutral mucins, Gordon-Sweet procedure for reticular fibers and Sirius stain for basal membrane and fibrous tissue.

The effective osmolarity pressure is mainly built up by the vehicle osmolarity. A titration graph was constructed with the osmolarity of the phosphate buffer varying between 0.06 and 0.30 mol. The vehicle osmolarity was then determined by the freeze point depression technique in a Halb-mikro osmometer (Knauer, Berlin). The vehicle did not contain any fixative and the results approximately represent the effective osmolarity within the fixed tissue. Thus the graph can be used to evaluate the desired vehicle osmolarity (Fig. 1).

The material from the invasive carcinomas was taken with a pair of biopsy forceps, especially designed for cervical tissue (STENDAHL and Stille-Werner, Stockholm; Fig. 2).

Results

From the cone material (CIS) it was concluded that the effective osmolarity should be at least 300 mmol/l and the number of fixative combinations was reduced to four for the invasive carcinoma specimens. With a hypo-osmolar vehicle (<250 mol/l) the tissue showed adequate staining contrast. There

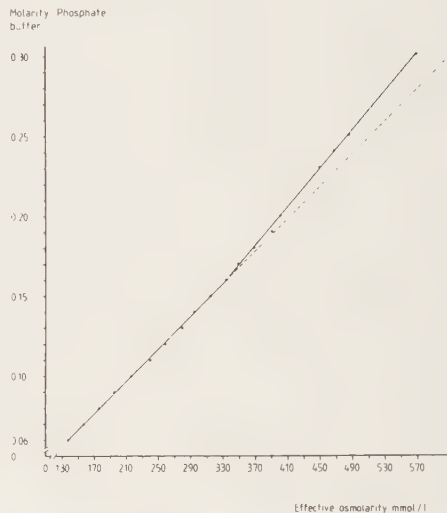


Fig. 1. Titration graph of the buffer vehicle molarity correlated with the effective osmolarity.

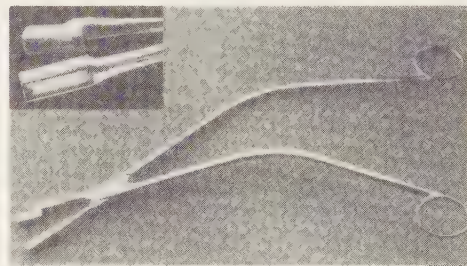


Fig. 2. The biopsy forceps specially designed for cervical tissue. Insert: The construction of the cutting mechanism (12.5 mm $\times$ 3.7 mm).

was, however, a hypotonic damage consisting of rarefaction of cytoplasmic organelles, cracking and swelling distortions as well as collapse of small vessels (Figs 3, 4).

Following fixation in hyperosmolar media (<350 mmol/l), the cells appeared shrunken with increased intercellular tissue spaces. The stainability was usually satisfactory but deteriorated with increasing osmolarity (<450 mmol/l). The tissue became stiff and more difficult to process with conventional embedding methods, and to cut.

The best result was achieved with an osmolarity

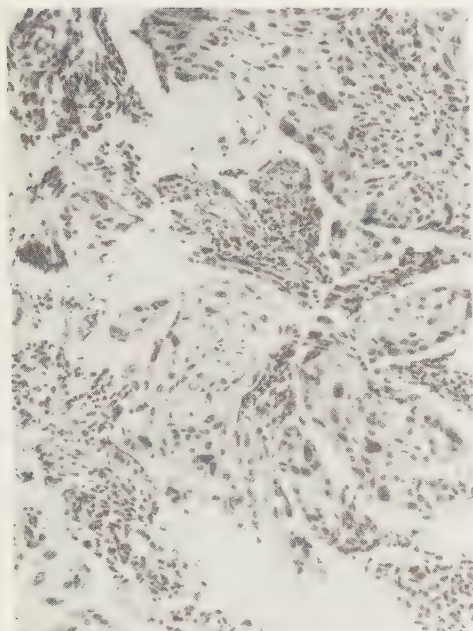


Fig. 3. Biopsy specimen taken with a conventional biopsy instrument and fixed in hypo-osmolar fixative. Adequate staining but cracking tissue damage making the evaluation difficult. HE×160.



Fig. 4. Biopsy specimen fixed in hypo-osmolar fixative showing rarefaction of cytoplasmic tissue giving an indistinct contrast between cells and nearly totally collapsed vessels within the stromal area (mid left area). HE×160.

of 0.17 mol phosphate buffer at pH 7.0 which gave an effective vehicle osmolarity of 350 mmol/l (Fig. 5).

The biopsy forceps designed for cervical tissue was superior to conventional ones (Fig. 2). At the same time both tissue volume and biopsy quality were improved after having adjusted the size of the cutting mechanism to 12.5 mm×3.7 mm (Fig. 5).

The titration graph for molarity of the phosphate buffer correlated well with the effective osmolarity as given in Fig. 1.

Discussion

The aim of tissue fixation is to maintain the original structure and thus make it possible to study the biopsy specimen unchanged from the moment it is fixed (HOPWOOD 1972). Autolysis and growth of bacteria and fungus should be prevented. There should be no reduction of substance and no changes

within the tissues and cellular volume and shape (SCHULTZ and KARLSSON 1965). No changes should appear in the relationship between different cell organelles and macromolecules and all molecules should remain at their original sites. Enzymatic activity and antigen properties should be restored (BRUNK & ERIKSSON 1972). No fixative can fulfill all these requirements as some of them are mutually incompatible (BRUNK & ERIKSSON).

Most modern fixative techniques utilize aldehyde fixation, usually glutaraldehyde or formaldehyde. Formaldehyde, with a molecular weight of 30, is, as a gas, easily dissolved in water. A saturated water solution will contain 37 to 38 per cent of formaldehyde and is termed formalin; 10 per cent formalin is thus 3.7 per cent formaldehyde.

Fixation artefacts, mainly cellular swelling, frequently render the histopathologic diagnosis of subtle abnormalities difficult (COLLINS et coll.). These artefacts may mimic pathologic alterations (AR-

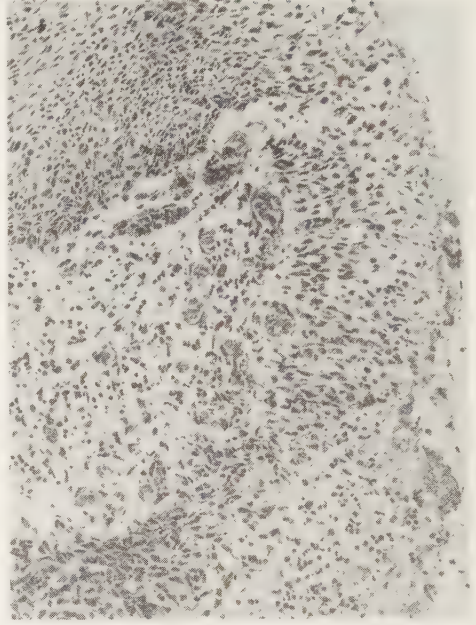
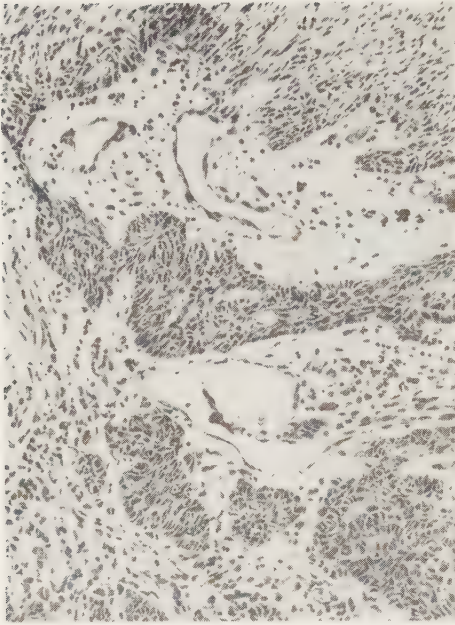


Fig. 5. Biopsy specimens from the same patient as in Fig. 4. The biopsy taken with the specially designed biopsy forceps, fixed in 0.17 mol phosphate buffer at pH 7.0 with 10 per cent formalin.

Excellent staining properties together with minimum of cracking and wide open lymph (a) and blood vessels (b). HE $\times 160$.

BORGH *et coll.*). Neutral buffered formalin has been recommended for routine fixation purposes but instead, usually diluted commercial formalin is used. In fixatives of this type the molarity of the salts constituting the buffer is often very low, resulting in a low effective osmolarity (BRUNK *et coll.*).

Cellular swelling is not adequately prevented and the effects of fixation are suboptimal with respect to the preservation of cellular details (BAHR *et coll.* 1957). In histopathologic biopsies this often results in section distortion and cracking, and collapse of fragile tubular or cystic structures, causing difficulties in the interpretation of tumour invasion into mesenchymal tissue and vessels.

The mechanism of size changes induced by fixation is not completely understood, and is complicated (HOPWOOD 1969, BEYER-BOON *et coll.*). It has long been known that the osmotic pressure varies in different cells and in different tumours (PALADE 1956). In tumour biopsies a phosphate buffer of 0.15 mol normally corresponds to an effective osmolarity of 310 mmol/l (BRUNK *et coll.*). At pres-

ent, it is generally agreed that the concentration of aldehyde in the fixative is of minor importance in comparison with the composition of the buffer and that the latter should have an effective osmolarity of approximately 300 mmol/l (ARBORGH *et coll.*, COLLINS *et coll.*).

The graph for molarity of the phosphate buffer correlated with the effective osmolarity (Fig. 1) and is in agreement with the results of MASER *et coll.* (1967). The relationship is not perfectly linear all over the measured range. The explanation for this is that the salt ions in the phosphate buffer protolyse in several steps and that a complete dissociation will not be achieved in higher concentrations. More ions have to be added in that range in order to achieve the appropriate effective osmolarity (Fig. 1, difference between dotted and curved lines).

In contrast to DAVENPORT & BALL, who searched for fixatives with a wider range, the present authors believe that instead of hoping to find an all-round fixative, the fixative (RUITENBERG *et coll.*) and the effective osmolarity of the vehicle (HOPWOOD 1969)

must probably be analysed for each organ and form of disease. It has been concluded that the concentration of 0.17 mol phosphate buffer at pH 7.0 with 10 per cent formalin, yielding an effective osmolality of 350 mmol/l, gave optimal fixation of specimens from squamous cell carcinoma of the uterine cervix with respect to staining properties and absence of cracking defects and shrinkage. The vessels remained open, facilitating the analysis of vascular invasion, which has proved to be the most relevant histopathologic prognostic parameter for cervical carcinomas (STENDAHL et coll. 1981). The fixative method is easy to apply, inexpensive and easily introduced into gynecologic oncologic practice. The specially designed biopsy forceps yielded large representative biopsy specimens with a minimum of pressure distortion.

SUMMARY

A histopathologic grading system for more reliable determination of the prognosis in individual patients with squamous cell carcinoma of the uterine cervix necessitates better sampling and fixation techniques. Fixation in 10 per cent formalin and 0.17 mol phosphate buffer at pH 7.0, with an effective osmolality of 350 mmol/l, gave optimal stainability, and minimized tissue shrinkage and sectioning artefacts. Vessels remained open, which facilitated the analysis of vascular invasion. A newly designed biopsy forceps yielded large and representative specimens with a minimum of pressure distortion.

ACKNOWLEDGEMENT

Financial support was received from Cancerföreningen i Stockholm.

Request for reprints: Dr Roger Willén, Department of Histopathology, University Hospital, S-22185 Lund, Sweden.

REFERENCES

- ARBORGH B., BELL P., BRUNK U. and COLLINS V. P.: The osmotic effect of glutaraldehyde during fixation. A transmission electron microscopy, scanning electron microscopy and cytochemical study. *J. Ultrastruct. Res.* 56 (1976), 339.
- BAHR G. F., BLOOM G. and FRIBERG U.: Volume changes of tissue in physiological fluids during fixation in osmium tetroxide or formaldehyde and during subsequent treatment. *Exp. Cell Res.* 12 (1957), 342.
- BEYER-BOON M. E., VAN DER VOORN-DEN HOLLANDER M. J. A., ARENTZ P. W., CORNELISSE C. J., SCHABERG A. and FOX C. H.: Effect of various routine cytopreparatory techniques on normal urothelial cells and their nuclei. *Acta path. microbiol. scand. Sect. A* 87 (1979), 63.
- BOYDE A. and VESELY P.: Comparison of fixation and drying procedures for preparation of some cultured cell lines for examination in the SEM. *In: Scanning electron microscopy*, p. 265. Edited by O. Johari and I. Corvin. ITT Research Institute, Illinois, 1972.
- BRUNK U. and ERIKSSON J. L. E.: The demonstration of acid phosphatase in in vitro cultured tissue cells studies on the significance of fixation, tonicity and permeability. *Histochem. J.* 4 (1972), 349.
- BELL P., COLLINS P., FORSBY N. and FREDRIKSSON B. A.: SEM of in vitro cultivated cells, osmotic effects during fixation. *In: Scanning electron microscopy*, p. 379. Edited by O. Johari and I. Corvin. ITT Research Institute, Illinois, 1975.
- COLLINS V. P., ARBORGH B. and BRUNK U.: A comparison of the effects of three widely used glutaraldehyde fixatives on cellular volume and structure. A TEM, SEM, volumetric and cytochemical study. *Acta path. microbiol. scand. Sect. A* 85 (1977), 157.
- DAVENPORT JR W. D. and BALL C. R.: Observations on the results of specific histochemical techniques and empirical staining methods on several tissue/organ types using a variety of fixing fluids. *Histopathology* 3 (1979), 321.
- HAYAT M. A.: Principles and techniques of electron microscopy. Vol. 1, p. 5. van Nostrand Reinhold, New York 1970.
- HOPWOOD D.: Fixatives and fixation. A review. *Histochem. J.* 1 (1969), 323.
- Theoretical and practical aspects of glutaraldehyde fixation. *Histochem. J.* 4 (1972), 267.
- MASER M. D., POWELL III T. E. and PHILPOTT C. W.: Relationships among pH, osmolality and concentration of fixative solutions. *Stain Technol.* 42 (1967), 175.
- PALADE G. E.: The fixation of tissue for electron microscopy. *In: Proc. 3rd Int. Congr. Electron microscopy*, p. 129. William Clowes, London, Beccles 1956.
- RUITENBERG E. J., GUSTOWSKA L., ELGERSMA A. and RUITENBERG H. M.: Effect of fixation on the light microscopical visualization of mast cells in the mucosa and connective tissue of the human duodenum. *Int. Arch. Allergy* 67 (1982), 233.
- SCHULTZ R. L. and KARLSSON U.: Fixation of the central nervous system for electron microscopy by aldehyde perfusion. II. Effect of osmolality, pH of perfusate and fixative concentration. *J. Ultrastruct. Res.* 12 (1965), 187.
- STENDAHL U., WILLÉN H. and WILLÉN R.: Invasive squamous cell carcinoma of the uterine cervix. I. Definition of parameters in a histopathologic malignancy grading system. *Acta radiol. Oncology* 19 (1980), 467.
- EKLUND G., WILLÉN H. and WILLÉN R.: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. *Acta radiol. Oncology* 18 (1979), 481.
- — — Invasive squamous cell carcinoma of the uterine cervix. III. A malignancy grading system for indication of prognosis after radiation therapy. *Acta radiol. Oncology* 20 (1981), 231.

FROM THE DEPARTMENT OF PATHOLOGY AND THE GYNECOLOGIC SECTION, DEPARTMENT OF ONCOLOGY,
UNIVERSITY HOSPITAL, S-22185 LUND, SWEDEN.

INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

VI. Prediction value of non-keratinizing, parakeratotic and orthokeratotic cell forms and clinical staging

H. WILLÉN, R. WILLÉN and U. STENDAHL

Several attempts have been made to adapt the treatment modalities to the histopathologic classification of tumour biopsies. Monofactorial systems based on cell differentiation have been used together with clinical staging, which for some time has proven to be of superior prognostic value. However, the additional information, if any, should be identified. According to REAGAN et coll. (1957), WENTZ & REAGAN (1959) and REAGAN & WENTZ (1967), invasive squamous cell carcinomas can be divided into large cell non-keratinizing, large cell keratinizing and small cell carcinoma. This classification has been adopted by the WHO (POULSEN et coll. 1975). The keratinizing large cell carcinomas were supposed to be derived from the squamous epithelium of the portio, large cell non-keratinizing carcinomas from metaplastic epithelium within the junctional zone and the small cell carcinomas from the reserve cells (WENTZ 1966). A significantly higher survival rate has been reported in patients with large cell non-keratinizing compared with large cell keratinizing or the small cell tumours. The latter had the poorest survival rate of all. These results from the group of REAGAN & WENTZ were confirmed by others (FINCK & DENK 1970, SIDHU et coll. 1970, NG & ATKIN 1973, SWAN & RODDICK 1973, VAN NAGELL et coll. 1977, HARDT et coll. 1982) both for irradiation and surgical treatment. This was in contrast to the results of FIELD et coll. (1964), GUNDERSEN et coll. (1974) and GOELLNER (1976). Except for

a preliminary report presented by STENDAHL et coll. (1979) reports from Scandinavia (LINELL & MANS-SON 1952, NØDSKOV-PEDERSEN 1971, JOHANSSON et coll. 1976, BEECHAM et coll. 1978) have not shown any difference in survival after sub-grouping into cell types. The contradictory results reported by using the definitions of REAGAN & WENTZ might be explained by deviations from the original definitions. The aim of the present report is: a) to discuss the definitions of the different cell types of cervical carcinoma, b) to relate them to the 10-year lethality rate (lethality/lethality + survival), c) to discuss the different keratinizing cell forms such as single cell keratinization, parakeratotic and orthokeratotic forms in a context of a continual spectrum of keratinization rather than special cell forms.

Material and Methods

The material consisted of 432 consecutive patients stages IB–IV with invasive squamous cell carcinoma of the uterine cervix classified and treated at the Department of Gynecologic Oncology, University Hospital, Uppsala, during the years 1967 to 1971. In the final analysis 3 patients were omitted due to revised diagnosis and 4 because of unsuitable biopsy material. Thirty-two patients died from inter-

Accepted for publication 24 August 1982.

Table

*Lethality rates within stages and cell types among 393 patients with carcinoma of the uterine cervix.
Per cent within parentheses*

Cell type	Stage				Total
	I	II	III	IV	
1. Large cell non-keratinizing	10/61 (16)	15/53 (28)	7/7 (100)	4/4 (100)	36/125 (28)
2P. Large cell parakeratotic	4/35 (11)	30/75 (40)	7/13 (54)	4/4 (100)	45/127 (35)
2K. Large cell keratotic	5/23 (22)	33/63 (52)	21/26 (81)	12/13 (92)	71/125 (57)
3. Small cell	1/10 (10)	2/5 (40)	— —	1/1 (100)	4/16 (25)
Total	19/128 (15)	78/196 (40)	35/46 (76)	21/22 (95)	156/393 (40)

current diseases. Thus 393 patients were included in the series. The age varied between 22 and 82 years. All patients were kept under observation until death or for at least 10 years. Staging was done according to the FIGO classification (1971).

Treatment. Intracavitary irradiation was carried out according to a modified Stockholm-method (KOTTMEIER 1964) with separate intrauterine and vaginal irradiators in two sessions at an interval of 3 weeks. The mean dose was 7500 mgh. External irradiation was given to two opposite pelvic fields with 8 MV roentgen rays (linear accelerator), 33 MV roentgen rays (Betatron) or ^{60}Co photons. The majority of patients received a parametrial dose of 40 Gy (mean dose) in 20 fractions usually over a period of 4 weeks (5 irradiations/week) and with central shielding. Individual dose planning was done for each patient. A detailed description of treatment modalities has been given previously (STENDAHL et coll. 1981). The treatment in all cases was thus similar and did not differ significantly between stages.

Histopathology

The estimations of histopathologic parameters were performed retrospectively on pretreatment biopsies and without knowledge of treatment and further course of the disease. The original slides were re-examined twice at an interval of at least 3 months. The result of the last examination was used. At the re-examination new sections, 5 µm

thick, were cut of the paraffin blocks. Both van Gieson and Hematoxylin-Eosin stained slides were used depending on the technique applied at the initial classification.

The definitions of REAGAN et coll. (1957) were carefully followed apart from additional sub-grouping into parakeratotic and orthokeratotic large cell keratinizing cell forms as follows:

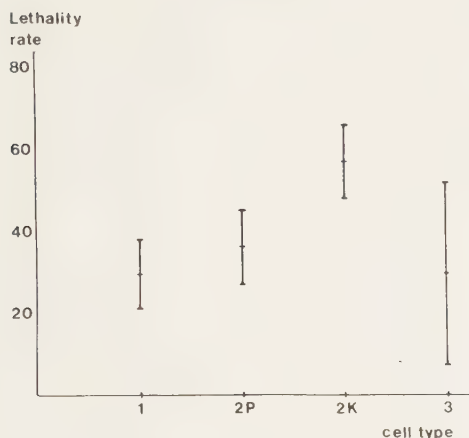
Type 1. Large cell non-keratinizing squamous carcinoma. No keratin production or single cells with intracytoplasmic keratin are referred to this group. A single superficial layer of parakeratosis can also be accepted in this group.

Type 2. Large cell keratinizing squamous carcinoma. 2P. Parakeratotic form: Keratinization without pearls, but with different amounts of remaining nuclei, often confined to the superficial area of the carcinoma and composed of several cell layers. 2K. Orthokeratotic form: All cases with light large cells and keratin pearls of both orthokeratotic and parakeratotic forms are referred to this group as are thick orthokeratotic desquamating types confined to the superficial area of the cancer.

Type 3. Small cell carcinoma. Small dark cells with an increased nuclear/cytoplasmic ratio and organophilic cytoplasm.

Results

The results appear in the Table. The lethality differed significantly between patients with type 3



Lethality rates for different cell types. Confidence limits are given for the 95 per cent level.

and type 2K ($p \leq 0.05$), which was higher in 2K. Corresponding figures for type 2K and type 1 were ($p \leq 0.001$) and for 2K and 2P ($p \leq 0.001$), respectively. No differences were found between cell types 1, 2P and 3. The confidence limits (95 per cent level) are given in the Figure. In patients with cell type 3 the small number of patients (16) gave a confidence interval between 0.07 and 0.52. Thus no firm conclusions can be drawn with respect to clinical outcome in that group.

Within the same stage there were no differences between types 2K and 3 (in contrast to the total material), nor between 1 and 2P. Between types 1 and 2K a significant increase ($p \leq 0.001$) in lethality rates was found in stage II. No such tendency was observed in stages I, III and IV.

Significant differences in lethality rates were found between clinical stages, and these were greater than those derived from cell type differentiation. Thus, clinical staging cannot be supplanted by this classification. After inclusion of clinical staging, cell typing is of additional prognostic value only in stage II. Possible causal connections between cell type differentiation and stage cannot be established in the present series.

Discussion

It is difficult to group according to the definitions of REAGAN & WENTZ. In spite of the precise defini-

tions given, 10 to 20 per cent of the patients will fall outside the 3 groups (GUNDERSEN et coll.). The difficulties of evaluation become obvious when 2 or 3 different cell types are present within the same biopsy. REAGAN & WENTZ classified according to the most differentiated component while FINCK & DENK did the opposite. BEECHAM et coll. used the predominant cell type and GUNDERSEN et coll. tried both the latter alternatives. It is not surprising that the results have been most varying. Both orthokeratotic and parakeratotic cell forms occur together with or without horn pearl-formation and single cell-keratinizing, thus providing for numerous combinations. It is often not possible to attain definite knowledge, in different reports, regarding how these intermediate forms have been grouped even when elaborate definitions have been given (NG & ATKIN, GUNDERSEN et coll.).

The normal cervical mucosa is not keratinized and in agreement with WENTZ & REAGAN the non-keratinizing large cell carcinoma was considered the most differentiated one because of its resemblance to the mature cells of normal stratified squamous mucosa. Keratinized cells are considered to represent an abnormal differentiation, producing only keratin fibers, a process ending up with the cells being unable to divide and consequently dying off (GLÜCKSMANN 1956). True cornification is rare in carcinoma of the cervix (LINELL & MANSSON), but as a rule the keratinization is associated with the development of a stratum granulosum, at least in isolated foci (BURGHARDT 1973, NG & ATKIN, BEECHAM et coll.). STÜPER (1953) differentiated the keratinizing horn pearl forming type from the parakeratotic one, the latter being often superficial and with only a keratinizing tendency. The parakeratotic form has also been differentiated from horn pearl forming types by SCHRÖDER (1922), MARTZLOFF (1923), CORDUA (1926), HUEPER & SCHMITZ (1927) and LAHM (1927). REAGAN & WENTZ were of the same opinion as GUNDERSEN et coll., that only a few pearls are required to classify a tumour as a keratinizing squamous cell carcinoma.

The complexity of the keratinizing process is not fully understood (ADAMS 1976, MATOLTSY 1976). A continual spectrum seems to exist from non-keratinizing to para-keratinizing to ortho-keratinizing epithelia rather than distinctly different cell types (CHEN & MEYER 1971, ADAMS). The amount of keratinization is reflected by the degree of packing and orientation of tonofilaments (ADAMS). Ribosomal and ly-

sosomal activity (MATOLTSY), persistence of gene function, DNA and RNA content (MATOLTSY, SUZUKI *et coll.* 1977), thickness of basal membrane (ADAMS), turn-over rate (HAMILTON & BLACKWOOD 1974) and mitosis rate (HALPRIN 1972) contribute. Enzyme levels and vitamin status (GLUCKSMANN, WOLFF-SCHREINER 1977, KRUPP & FRINK 1978), oestrogen level (GLÜCKSMANN, LINDHOLM 1975), temperature (INDO & MIYAJI 1979) and the stromal component surrounding the malignant cells (ADAMS, GREEN *et coll.* 1977) also play a role. The water content of the cell (ADAMS), inflammation (HUGOSON *et coll.* 1971, ADAMS) and the natural degradation process which takes place within the keratocyte including keratohyaline activity and production of sulphur-rich proteins (MATOLTSY) may also interact.

The present series supports the thesis that the orthokeratotic cell type has the highest lethality rate and that the large cell non-keratinizing one has the lowest. As a single prognostic parameter the classification of REAGAN *et coll.* does not need to be changed with respect to definitions. However, if used clinically to predict the outcome for patients it has a limited value. In combination with clinical staging, it will have no additional value apart from a limited number of patients in stage II.

The reason why the keratinizing cell type of carcinomas has a worse prognosis compared with non-keratinizing large cell forms is obscure. Two main arguments have been put forward: 1) A real biologic difference exists (REAGAN *et coll.*, WENTZ & REAGAN) with a different genesis and localization (WENTZ, VAN NAGELL *et coll.*). Several reports on patients treated with surgery support this view (HUEPER 1928, SCHÜLLER 1952, SIDHU *et coll.*). Others, however (GOELLNER, VAN NAGELL *et coll.*), could not find any difference in surgically treated patients. In this context the view of ADAMS, that keratinization is a question of degree of keratinization rather than separate cell types, should be mentioned. 2) According to GILMOUR *et coll.* (1949), REAGAN *et coll.*, MILLER *et coll.* (1959), and GUNDERSEN *et coll.*, radiation therapy does not influence the different cell types in a similar way. Following modern radiation therapy no differences in treatment results should occur between different cell types (SHERMAN 1961, SCOTT *et coll.* 1966, BOURNE & MEAD 1968, GUNDERSEN *et coll.*). In the present series the treatment given the various groups was similar. Considering the fact that these

patients were followed for up to 15 years it should be pointed out that neither treatment modality nor results have changed significantly during the past two decades. The different survival rates may partly be explained by the fact that DNA, RNA and other sub-cellular structures are still susceptible to irradiation in the non-keratotic and parakeratotic cell forms. In contrast, these sub-cellular structures are sparser in the orthokeratotic cell form.

In a multifactorial histopathologic classification of invasive squamous cell carcinoma of the uterine cervix presented previously, the cell type was one parameter of eight. Several other parameters had more prognostic information with respect to survival, especially those parameters representing the tumour-host relationship (mode of invasion, stage of invasion, vascular invasion and host-cellular response; STENDAHL *et coll.* 1979, 1980, 1981). However, any additional information which can be obtained from a single parameter such as cell type differentiation is valuable but should be extracted and be compared with other predictive parameters. In the present series, cell type differentiation by itself had a prognostic value which was subordinate to that from clinical staging.

The difference in lethality rates between large cell orthokeratotic or horn-pearl forming, and large cell non-keratinizing in stage II, affected 32 per cent of all the patients. Although highly significant the difference in specificity will have little consequence with respect to treatment.

Before a new method is introduced into practice its clinical significance should be established (RINGSTED *et coll.* 1978).

SUMMARY

In 393 patients with invasive squamous cell carcinoma of the uterine cervix stages I to IV the predictive value of the histopathologic classification of REAGAN & WENTZ (differentiation into cell type) was analysed in relation to 10-year lethality rate. Patients with large cell horn-pearl forming and orthokeratinizing tumours had the poorest, and those with large cell non-keratinizing the best prognosis. Large cell parakeratotic tumours did not differ significantly in prognosis compared with the non-keratinizing cell form. When clinical staging was included the prediction value of the histopathologic classification disappeared except in stage II. From a clinical point of view this additional prognostic information will have little practical consequences.

ACKNOWLEDGEMENTS

Financial support was received from Stockholms Cancerförening. The authors wish to express their gratitude to Prof. Stig Sténson for cooperation.

Request for reprints: Docent Roger Willén, Department of Pathology, University Hospital, S-221 85 Lund, Sweden.

REFERENCES

- ADAMS D.: Keratinization of the oral epithelium. *Ann. roy. Coll. Surg. Engl.* 58 (1976), 351.
- BEECHAM J. B., HALVORSEN T. and KOLBENSTVEDT A.: Histologic classification, lymph node metastases and patient survival in stage I B cervical carcinoma. An analysis of 245 uniformly treated cases. *Gynecol. Oncol.* 6 (1978), 95.
- BOURNE R. G. and MEAD K. W.: A proposed method of selecting patients with carcinoma of cervix for radiotherapy or surgery. *Radiology* 90 (1968), 139.
- BURGHARDT E.: Early histological diagnosis of cervical cancer. *Textbook and atlas.* Georg Thieme Verlag, Stuttgart 1973.
- CHEN S. Y. and MEYER J.: Regional differences in tonofilament and keratohyaline granules. *In:* Current concepts of the histology of the oral mucosa, p. 114. Edited by C. A. Squier and J. Meyer. Charles C. Thomas, Springfield, Illinois 1971.
- CORDUA R.: Die Morphologie der Kollum-Karzinome des Uterus als Grundlage ihrer Beurteilung für die Strahlenempfindlichkeit. *Strahlentherapie* 22 (1926), 689.
- FIELD C. A., DOCKERTY M. and SYMONDS R. F.: Small cell cancer of the cervix. *Amer. J. Obstet. Gynec.* 88 (1964), 447.
- FIGO-Fédération Internationale de Gynécologie et Obstétrique: Classification and staging of malignant tumours in the female pelvis. *Acta obstet. gynec. scand.* 50 (1971), 1.
- FINCK F. M. and DENK M.: Cervical carcinoma. Relationship between histology and survival following radiation therapy. *Obstet. and Gynec.* 35 (1970), 339.
- GILMOUR M., GLUCKSMANN A. and SPEAR F. G.: The influence of tumour histology, duration of symptoms and age of patients on the radiocurability of cervix tumours. *Brit. J. Radiol.* 22 (1949), 90.
- GLUCKSMANN A.: The influence of systemic factors on the differentiation and radiocurability of cervical cancers. *Brit. J. Radiol.* 29 (1956), 483.
- GOELLNER J. R.: Carcinoma of the cervix. Clinicopathologic correlation of 196 cases. *Amer. J. clin. Path.* 66 (1976), 775.
- GREEN H., RHEINWALD J. G. and SUN T. T.: Properties of an epithelial cell type in culture. The epidermal keratinocyte and its dependence on products of the fibroblast. *Prog. Clin. Biol. Res.* 17 (1977), 493.
- GUNDERSEN L. L., WEEMS W. S., HEBERTSON R. M. and PLENK H. P.: Correlation of histopathology with clinical results following radiation therapy for carcinoma of the cervix. *Amer. J. Roentgenol.* 120 (1974), 74.
- HALPRIN K. M.: Epidermal 'turnover time'. A re-examination. *Brit. J. Dermatol.* 86 (1972), 14.
- HAMILTON I. A. and BLACKWOOD H. J. J.: Cell renewal of oral mucosal epithelium of the rat. *J. Anat.* 117 (1974), 313.
- HARDT N., VAN NAGELL J. R., HANSON M., DONALDSON E., YONEDA J. and MARUYAMA Y.: Radiation-induced tumour regression as a prognostic factor in patients with invasive cervical cancer. *Cancer* 49 (1982), 35.
- HUEPER W. C.: The relation of the histological structure to the prognosis of the carcinomas of the uterine cervix. *Surg. Gynec. Obstet.* 47 (1928), 502.
- und SCHMITZ H.: Der histologische Malignitäts-Index und seine Bedeutung für Prognose und Behandlung der Cervixcarcinome des Uterus. *Strahlentherapie* 24 (1927), 660.
- HUGOSON A., WINBERG E. and ANGSTRÖM T.: Cytologic findings in vaginal and oral smears from pregnant women. *Odont. Revy* 22 (1971), 145.
- INDO K. and MIYAJI H.: Qualitative changes in the biologic characteristics of cultured fetal rat keratinization epidermal cell during the process of malignant transformation after benzo (a) pyrene treatment. *J. nat. Cancer Inst.* 63 (1979), 1017.
- JOHANSSON O., JOHANSSON J. E., LINDBERG L. G. and SYDSJÖ A.: Prognosis, recurrences and metastases correlated to histologic cell type in carcinoma of the uterine cervix. *Acta obstet. gynec. scand.* 55 (1976), 255.
- KOTTMEIER H. L.: Surgical and radiation treatment of invasive carcinoma of the uterine cervix. Experience by the current individualized Stockholm method. *Acta obstet. gynec. scand.* (1964) Suppl. No. 2, p. 43.
- KRUPP P. P. and FRINK R.: Effects of vitamin A deficiency on ultimobranchial cysts in the thyroid gland. An electron microscopic study. *Cell. Tiss. Res.* 190 (1978), 181.
- LAHM W.: Die Bedeutung der mikroskopischen Untersuchung für die Behandlung und Prognose des Collum-Karzinoms. *Ergebn. med. Strahlenforsch.* 1 (1927), 556.
- LINDHOLM K.: A cytological study of the keratinization of palatal mucosa in young men and women. *Proc. Finn. Dent. Soc.* 71 (1975), 84.
- LINELL F. and MÄNSSON B.: The prognostic value of histologic grading of carcinoma of the cervix uteri. *Acta radiol.* 38 (1952), 219.
- MARTZLOFF K. H.: Carcinoma of cervix uteri. Pathological and clinical study with particular reference to relative malignancy of neoplastic process as indicated by predominant type of cancer cell. *Bull. Johns Hopk. Hosp.* 34 (1923), 141.
- MATOLTSY A. G.: Keratinization. *J. invest. Derm.* 67 (1976), 20.
- MILLER N. F., HINERMAN D. L., RILEY G. M., LUDOVICI P. P., GOSLING J. R., CHRISTIAN R. T. and HALL D. F.: The nature of cervix cancer. *Obstet. and Gynec.* 14 (1959), 703.
- VAN NAGELL J. R., DONALDSON E. S., PARKER J. C., VAN DYKE A. H. and WOOD E. G.: The prognostic significance of cell type and lesion size in patients with cervical cancer treated by radical surgery. *Gynecol. Oncol.* 5 (1977), 142.
- NG A. B. P. and ATKIN N. B.: Histological cell type and

- DNA value in the prognosis of squamous cell cancer of uterine cervix. *Brit. J. Cancer* 28 (1973), 322.
- NØDSKOV-PEDERSEN S.: Degree of malignancy of cancer involving the cervix uteri, judged on the basis of clinical stage, histology, size of nuclei and content of DNA. *Acta path. microbiol. scand. A* 79 (1971), 617.
- POULSEN H. E., TAYLOR C. W. and SOBIN L. H.: Histological typing of female genital tract tumours. *In: International histological classification of tumours*. No. 13. WHO, Geneva 1975.
- REAGAN J. W. and WENTZ W. B.: Genesis of carcinoma of uterine cervix. *Clin. Obstet. Gynecol.* 10 (1967), 883.
- HARMONIC M. J. and WENTZ W. B.: Analytical study of cells in cervical squamous cell carcinoma. *Lab. Invest.* 6 (1957), 241.
- RINGSTED J., AMTRUP F., ASKLUND C., BAUNSGAARD P., CHRISTENSEN H. E., HANSEN L., JAKOBSEN C., JENSEN N. K., MOESNER J., RASMUSSEN J., REINTOFT I., ROTSCHAU J., STARKLINT H., THOMMESEN N. and VRANG J.: Reliability of histo-pathological diagnosis of squamous epithelial changes of the uterine cervix. *Acta path. microbiol. scand. A* 86 (1978), 273.
- SCHRÖDER R.: *Lehrbuch der Gynäkologie*. F. C. W. Vogel, Leipzig 1922, p. 548.
- SCHÜLLER E.: Beziehungen des histologischen Tumortypus des Collumcarcinoms zur Dauerheilung durch Operation und seine Bedeutung für Prognose und Therapie dieser Erkrankung. *Arch. Gynäk.* 181 (1952), 360.
- SCOTT R. M., BRIZEL H. E. and WETZELBERGER C.: Etiology of treatment failures in early stage carcinoma of cervix. *Amer. J. Roentgenol.* 96 (1966), 565.
- SHERMAN A. I.: Study of radiation failures and role of radioresistance in treatment of cancer of cervix. *Amer. J. Roentgenol.* 85 (1961), 466.
- SIDHU G. S., KOSS L. G. and BARBER H. R. K.: Relation of histologic factors to response of stage I epidermoid carcinoma of the cervix to surgical treatment. Analysis of 115 patients. *Obstet. and Gynec.* 35 (1970), 329.
- STENDAHL U., WILLÉN H. and WILLÉN R.: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. *Acta radiol. Oncology* 18 (1979), 481.
- — — Invasive squamous cell carcinoma of the uterine cervix. I. Definition of parameters in a histopathologic malignancy grading system. *Acta radiol. Oncology* 19 (1980), 467.
- EKLUND G., WILLÉN H. and WILLÉN R.: Invasive squamous cell carcinoma of the uterine cervix. III. A malignancy grading system for indication of prognosis after radiation therapy. *Acta radiol. Oncology* 20 (1981), 231.
- STÜPER P.: Über Beziehungen zwischen histologischer Struktur und Heilung der Kollumkarzinome. I. Mitteilung. Untersuchungen über die histologische Klassifizierung nach Pendl. *Strahlentherapie* 92 (1953), 89.
- SUZUKI H., FUKUYAMA K. and EPSTEIN W. L.: Changes in nuclear DNA and RNA during epidermal keratinization. *Cell. Tiss. Res.* 184 (1977), 155.
- SWAN D. S. and RODDICK J. W.: A clinical-pathological correlation of cell type classification for cervical cancer. *Amer. J. Obstet. Gynec.* 116 (1973), 666.
- WENTZ W. B.: Histogenesis of squamous cell carcinoma of the uterine cervix. *In: New concepts of gynecological oncology*, p. 51. Edited by G. C. Lewis, W. B. Wentz and R. M. Jaffe. Davies, Philadelphia 1966.
- and REAGAN J. W.: Survival in cervical cancer with respect to cell type. *Cancer* 12 (1959), 384.
- WOLFF-SCHREINER E. C.: Ultrastructural cytochemistry of the epidermis. *Int. J. Dermatol.* 16 (1977), 77.

SURVIVAL AND MALIGNANCY GRADING OF INVASIVE SQUAMOUS CELL
CARCINOMA OF THE UTERINE CERVIX TREATED BY IRRADIATION IN LUND
1969-1970

^xGunnar Eklund, ^μJohn-Erik Johnsson, ^øUlf Stendahl,
^μClaes Tropé, and ^{*}Helena Willén ^{**}

^xDepartment of Cancer Epidemiology, Radiumhemmet, Karolinska
Hospital, Stockholm

^μGynecologic Section, Department of Oncology, and

^{*}Department of Pathology, University Hospital, Lund and

^øDepartment of Oncology, Division of Gynecologic Oncology,
Akademiska Sjukhuset, Uppsala, Sweden.

^{**}The authors are named in the alphabetical order.

Reprint request to Dr Helena Willén
Department of Pathology
University Hospital
S-221 85 Lund, Sweden.

Acknowledgement: Financial support was received from the
University and University Hospital, Lund and John and Augusta
Persson's Foundation.

INTRODUCTION

A multifactorial histopathologic malignancy grading system (MGS) in patients with invasive squamous cell carcinoma of the uterine cervix has been shown to have prognostic value in earlier studies (Stendahl et coll. 1979, 1981 a, 1983).

Our aim is to correlate various predictive factors to the long term prognosis of the patients with invasive squamous cell carcinoma of the uterine cervix stage I A - II B treated by irradiation at the gynecologic section, Department of Oncology, University Hospital of Lund during the period 1969-1970. The MGS, histological classifications, and clinical data were studied in detail with respect to the 10-year survival/lethality rate.

MATERIAL

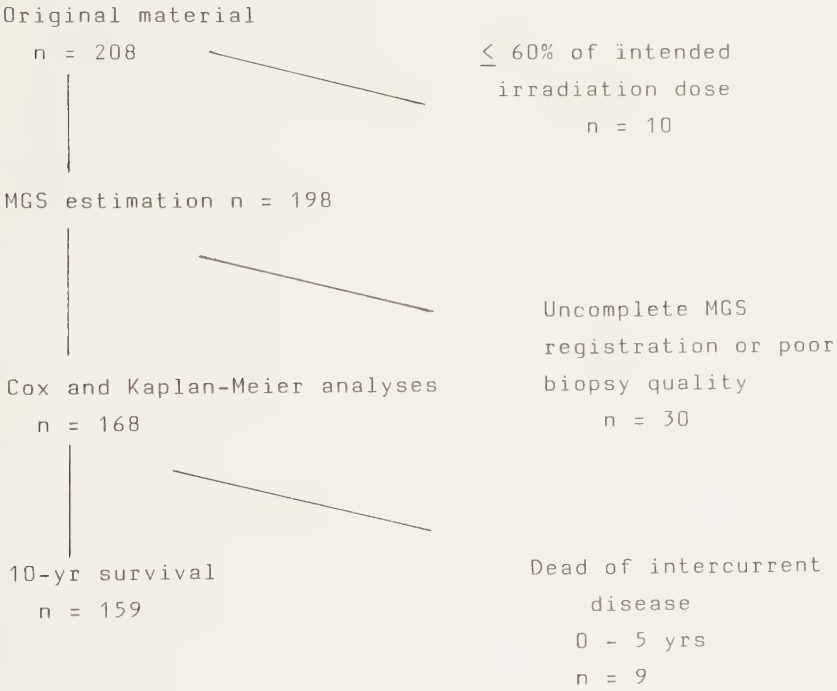
The material consisted of 208 patients with invasive squamous cell carcinoma of the uterine cervix stages IA to IIB treated during the years 1969 to 1970. The boundary between stage I A and I B in cases where cone biopsy was performed has been established according to Frick et coll. 1963. Twelve patients were advanced from stage IA to IB after the histopathologic reclassification.

Ten patients who had received less than 60 per cent of the intended irradiation dose in point A (Johnsson 1977) were excluded. No attention was otherwise paid to the tumour dose. Patients who exclusively received external irradiation were included in the study. Thus, the material was reduced to 198 patients (Fig 1).

Finally, when the histopathologic malignancy grading was retrospectively performed, 30 patients were expelled who could not be evaluated with respect to all histologic parameters or

Figure 1

Patients with invasive squamous cell carcinoma of the uterine cervix.



Material reduction process in present study (n = 208;
n = 159).

had a biopsy of poor quality, leaving 168 patients in the study. The material reduction process is illustrated in Fig 1.

Correction was made for patients dead from intercurrent disease within 5 years after onset of disease (9 patients) n=159. The follow-up was 10 years. The mean age at admission was 49 years. Stage distribution of the material is presented in Table 1.

Table 1

Clinical stage

Distribution of patients by clinical stage (n = 159).

Stage	<u>Number of patients</u>
IA	23
IB	58
IIA	53
IIB	25
Total	159

METHODS

Pathology

All biopsies were originally classified according to the Ackerman & Del Regato scheme (1947) into highly, moderately differentiated and anaplastic carcinoma. A certain number of cases remained "unclassified" in the primary report.

Reagan and Wentz (1967) cell type subclassification of the tumours into large cell non-keratinizing, large cell keratinizing and small cell carcinoma was also used. The predictors are listed in Table 2.

In all cases the pathologic reclassification with the malignancy grading system (MGS) was performed on the pretreatment biopsies

Table 2

List of predictors in the study

	Range	Number of classes	Comments
Year of birth	1885-1950	7	
Admission year	1969-1970	2	
Stage	I A -II B	4	(see Table 2)
Reagan & Wentz classification		4	(see text)
Ackerman & Del Regato		4	(see Table 6)
External irradiation	-65 Gy	6	see Kottmeier
" point A	-70 Gy	7	1964, Johnsson
Intracavitary irradiation A1	-35 Gy	4	1977 and Jonsson
" " A2	-33 Gy	7	& Nordberg 1973
Max. portio	" -160 Gy	3	
P ₁ structure	1p - 3p	3	
P ₂ cell differentiation	"	3	
P ₃ nuclear polymorphism	"	3	
P ₄ mitoses	"	3	
P ₅ mode of invasion	"	3	
P ₆ stage of invasion	"	3	
P ₇ vascular invasion	"	3	
P ₈ lymphoplasmocytic infiltration	"	3	
MGS	8p -24p	10	(total sum of score P ₁ -P ₈)
Biopsy quality		3	(poor quality excluded)
Biopsy depth	-1 cm	10	

without knowledge of the clinical stages, the treatment or the further course of the disease. The tumour cell population and the tumour-host relationship were considered separately. The evaluation of the tumour cell population was based upon the grading of tumour structure (P_1), cell differentiation (P_2), nuclear polymorphism (P_3) and the frequency of mitotic figures (P_4) on a scale of 1 to 3. The tumour-host relationship was graded on a similar scale, by evaluation of the mode (P_5) and the stage of invasion (P_6), vascular invasion (P_7) and the degree of lymphoplasmocytic infiltration (P_8). These eight morphologic parameters constituted the MGS which could thus range from 8 to 24 points (Stendahl et coll. 1979, 1980, 1983).

Treatment

The patients were treated according to a modified "Stockholm method" (Kottmeier 1964, Johnsson 1977). Early stage I A patients were only given two intracavitary treatments three weeks apart with a calculated dose in point A of 55 Gy. Advanced stage I A, I B and II patients were given primary external irradiation to the content of the true pelvis without any central shielding using ^{60}Co or 33 MV fotons. The absorbed dose in the true pelvis has been 33 Gy $\pm$ 10% given in 17 - 18 fractions and 44 Gy $\pm$ 10% in 23 - 24 fractions for stage I B and II respectively using two opposing fields; one fraction a day, 5 fractions a week. Intracavitary treatment of the primary tumour was given three weeks after completion of external irradiation (Johnsson & Nordberg 1973). The calculated dose in point A was 25 - 35 Gy. In cases with anatomical difficulties due to lack of satisfactory applicators only external treatment has been given. Calculated absorbed dose in the primary tumour was 64 Gy $\pm$ 3%. Fourfield-technique was used with two thirds of the absorbed dose given in a first series followed by an intermission of 2 - 3 weeks. (Mean delivered total absorbed dose in point A (n = 198 patients) has varied from 50 - 65 Gy depending on the stages). The studied predictors are given in Table 1.

Statistics

Significance analysis was made for each factor in order to investigate significant correlation to the 10-year survival (χ^2 -test or Fisher's exact test).

Estimation and significance testing in the regression analyses were made with the help of the program BMDP2R for linear regression stepwise analysis and BMDPLR for logistic regression analysis and for Cox regression program BMDP2L (Dixon 1981). The latter was performed to smooth the relation between survival rate and the MGS.

AID (Automatic Interaction Detector) analysis, according to the Osiris (Osiris III 1973, Rattenbury and Pelletier 1974) package was performed. Further, mass-significance analysis according to Eklund & Gavatin (1972) was performed. The reproducibility criterion in the AID analysis was specified as 0.4 and the minimal number of observation was 10. All the variables studied, except for classification according to Ackerman & Del Regato and into cell type (Reagan and Wentz) were monotone. The classification according to Ackerman & Del Regato was included as a free variable, because there were many unclassified cases in the primary histopathologic report.

Survival curves according to Kaplan & Meier were carried out (BMDP1L). Standard errors and confidence intervals were used as they were presented in the respective programs. Significance analyses of differences between correlation coefficients were performed as in Stendahl et coll. (1981 a).

RESULTS

Intercurrent deaths The 10-year S/L rate of 168 patients which could be classified with the MGS is shown in Table 3. 15 women died of intercurrent disease during the 10-year period. The expected lethality according to the life table for the period

1971-1975 in Sweden was found to be 16.6 (Statistical abstract of Sweden 1977), which rather well agrees with the number of incurrently dead cases (n=15). If the increased lethality due to the cancer disease (27 cases in the 10-year period) was considered, the expected number of deaths decreased to 15.5.

Table 3

Dead, censored and living patients (n=168).

Malignancy point grade MGS	Death in disease		Censored due to		Alive at 10 yr follow up	Total
	0-5 yr	5-10 yr	intercurrent disease			
			0-5 yr	5-10 yr		
< 12p	0	0	1	4	33	38
13-15p	4	5	6	2	79	96
> 16p	16	2	2	0	14	34
Total	20	7	9	6	126	168

10-year survival/lethality of different factors

The 10-year S/L rate of 159 patients in stage IA to IIB is shown in Table 4. There was a lower 10-year survival rate in between stage II B and stage I A + I B ($p < 0.01$). Further, the difference between II A and I A was significant ($p < 0.05$).

Table 5 shows the age distribution and the 10-year survival rates in different age groups. There is no significant difference between age groups with respect to the 10-year survival.

Table 4

10-year survival/lethality (S/L) rates in stages IA to IIB
(n=159).

Stage	Survival rate in per cent	(n)
IA	100	(23)
IB	88	(58)
IIA	77	(53)
IIB	68	(25)

Table 5

Age. 10-year survival rates in per cent in various age classes
(n=159).

Age at diagnosis (yrs)	per cent	(n)
20-39	89	(35)
40-49	81	(48)
50-59	83	(47)
60-	79	(29)

Table 6 illustrates the 10-year survival in the histological classification according to Ackerman & Del Regato in the primary histopathologic report. The highly differentiated carcinomas showed a better 10-year prognosis than the anaplastic ones ($p < 0.01$).

For differentiation into cell type (Reagan and Wentz) the survival rate was 82 per cent (n=29) in large cell non-keratinized carcinoma, 84 per cent (n=123) in large cell keratinized carcinoma and 43 per cent (n=7) in small cell carcinomas. The group of small cell carcinoma was too small to be conclusive for a prognostic comparison.

Table 7

The distribution of patients and survival at the 5- and 10-year follow up with respect to MGS points.

Malignancy point value MGS	Total number of cases		Survival	
	5-year	10-year	5 year	10 year
-10	6	6	6	6
11	9	9	9	9
12	23	22	23	22
13	27	27	26	26
14	33	30	32	26
15*	33	33	31	29
16*	17	17	11	10
17	9	8	5	3
18	3	3	1	1
19-	4	4	0	0
	163	159	143	132

* There was a highly significant difference in the 5- and 10-year survival between the patients with 15 and 16 points, χ^2 equals 5.5 and 7.1 respectively.

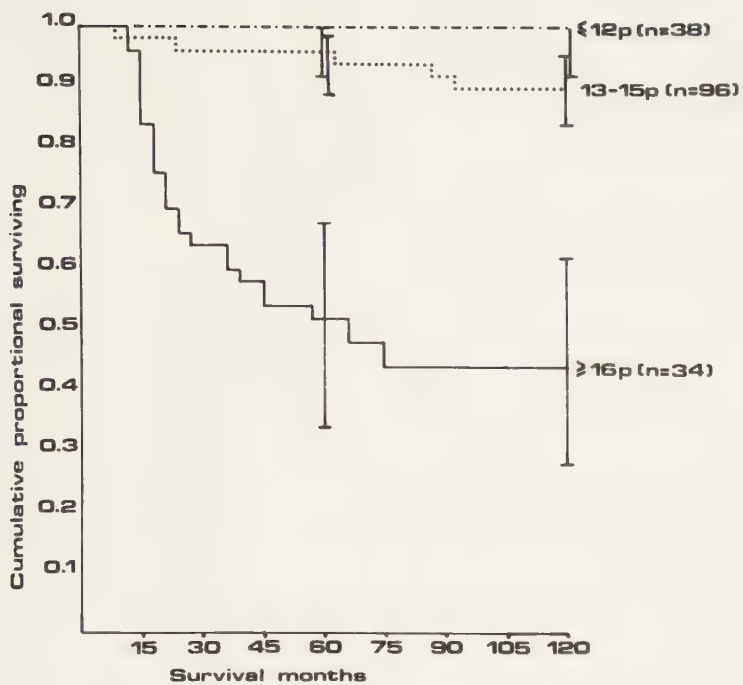


Fig 2.
Survival curves for three MGS classes (8 - 24 p). The
95% confidence interval for the 5- and 10-year survival.

Table 6

Histological classification (Ackerman & Del Regato).

Histological differentiation	10-year survival	
	per cent	n
high	97	(32)
moderate	82	(49)
anaplastic	72	(47)
"unclassified"	87	(31)

Admission year showed no significant difference in the relation to the S/L rate.

The number of lethal cases in various MGS-groups at the 5-, 8- and 10-year follow up is shown in Table 3. The survival rates are presented in survival curves according to Kaplan and Meier (Fig 2). The material is divided in Table 3 and Fig 2 into three categories:

1. low point MGS score patients (≤ 12 P) who all (100%) survived the 10-year follow up, 95% confidence interval (94, 100%).
2. a middle group of 13-15 points with 90% survival at 10-year follow up, confidence interval (84%, 96%).
3. high point MGS score patients (16 and more points) with 45% survival at 10-year follow up, confidence interval (28%, 62%). The 95% confidence intervals at the 5- and 10-years follow up are given in figure (2).

The patients with the high point MGS score showed significantly lower ($p < 0.001$) survival than both other groups at both the 5- and 10-year follow up. There was a significant difference between the high point score group and the low point score group. Particularly remarkable is the low lethality risk of the low MGS point score patients. The lower confidence borderline is 91% survival at 10 years.

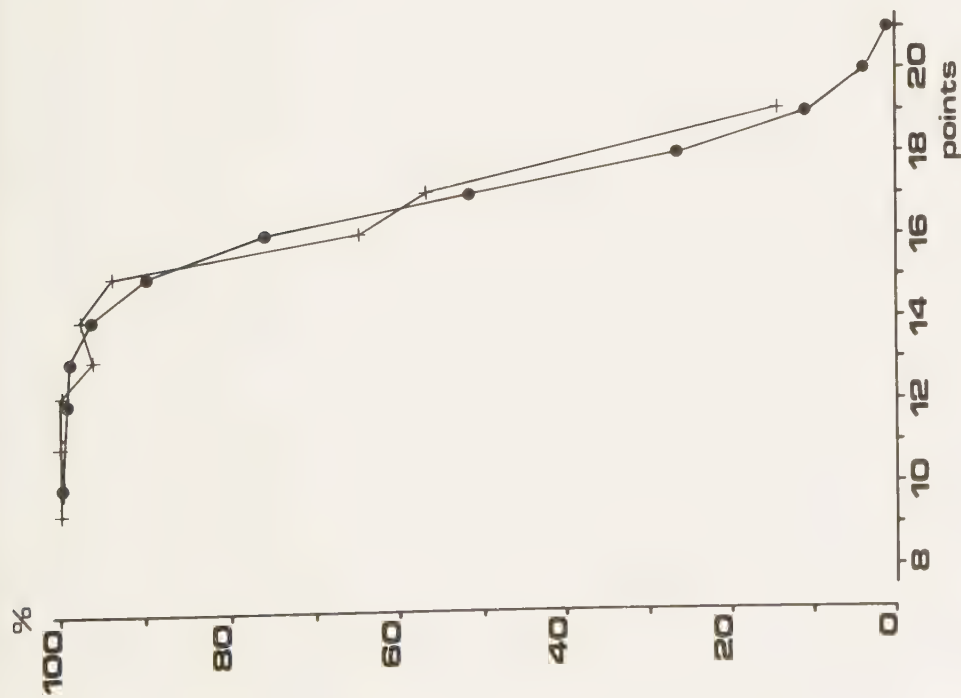


Fig. 3. Observed (x) and predicted (o) survival rates obtained by logistic regression analysis at the 5-year control (163 patients) in relation to MGS.

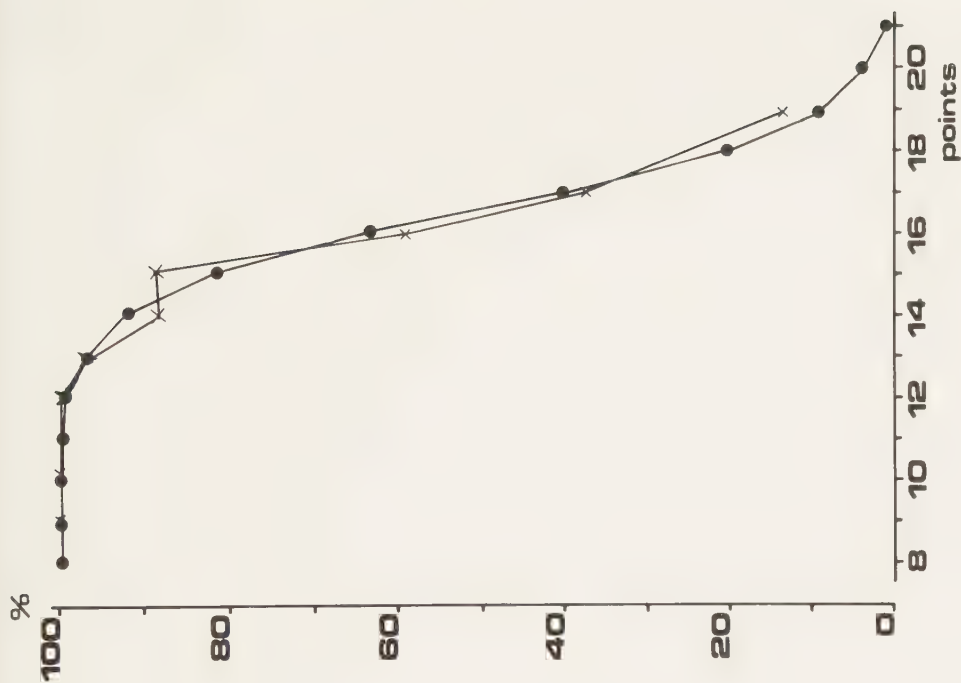


Fig. 4. Observed (x) and predicted (o) survival rates obtained by logistic regression analysis at the 10-year control (159 patients) in relation to MGS.

In table 7 the distribution of patients and of survivors is shown at the 5- and 10-year follow up, with respect to MGS points. From the table it is possible to see the difference between the two follow ups. It is evident that the lethality is higher during the first 5 years than during the second 5 years. Further, it is striking that the survival rate is higher among patients with a lower MGS score. This is true for both the 5- and 10-year follow up. The MGS results in relation to the 5- and 10-year survival show a significant difference in survival between patients with 15 and 16 points ($p < 0.001$). With increasing MGS value the prognosis deteriorated significantly ($p < 0.001$). The patients with a low score had an extremely good prognosis.

The relation between the MGS score values and survival is illustrated in Figure 3 and 4. The observed survival rates at 5- and 10-year are given in the figures as well as smoothed logistic regression curves. The goodness of fit between the observed and predicted curves is striking.

For the different items and their grade distribution and the 10-year survival rates are shown in Table 8. There were significant correlations between grade and the 10-year survival for most of the items; particularly strongly significant was the correlation between the vascular invasion (P_7) and the host-cellular response (P_8) and survival ($p < 0.001$). However, there were no significant correlations between survival and the differentiation into cell type (P_2) or mitosis (P_4). Almost always, an increasing MGS score gave a poorer 10-year prognosis.

Multivariate analyses of the prognosis

All results show that the MGS is strongly correlated to the survival. When each individual factor is analyzed separately, several with a significant correlation to the 10-year survival emerge; vascular invasion (P_7) and tumour-host cellular response (P_8). The histological classification according to Ackerman & Del Regato and FIGO staging were also significant

Table 8

10-year survival rate in different grades of parameters (n=159).

Parameter	grade 1		grade 2		grade 3	
P1 structure	86%	(144)	53%	(15)		
P2 differentiation into cell type	87%	(113)	77%	(43)	33%	(3)
P3 nuclear polymorphism	84%	(55)	92%	(59)	71%	(45)
P4 mitosis	83%	(24)	85%	(84)	80%	(51)
P5 mode of invasion	86%	(49)	85%	(100)	50%	(10)
P6 stage of invasion	100%	(16)	98%	(42)	74%	(101)
P7 vascular invasion	91%	(92)	86%	(42)	48%	(25)
P8 cellular response	93%	(83)	79%	(53)	57%	(23)

prognostic factors.

Multiple linear stepwise regression analysis, AID-analysis and Cox regression analysis have been performed in order to test if the correlation remains when different variables are analyzed simultaneously.

In a multiple linear stepwise regression analysis all factors presented in Table 2 candidated (Table 9 a, b). If the MGS (Table 9 a) was included it became the dominating variable. A one point increase in MGS corresponds approximately to a 9 per cent decrease in survival. Figure 3 and 4 based on logistic regression analysis, indicate that the sigmoid curve ought to be preferred to the linear regression.

If the MGS was excluded from the analysis (Table 9 b) vascular invasion (P_7) was the strongest variable together with host-cellular response (P_8) followed by structure (P_1) and stage II B (coded 1, the I A - II A coded 0) which showed the highest regression coefficients. The FIGO staging, treatment, histological classification into cell type or according to Ackerman & Del Regato or other single MGS items (P_1 , P_2 etc.) did not have the prognostic capacity of the MGS.

The regression analysis was supplemented by an AID analysis. There again all factors candidated. Figure 5 demonstrates only the factors which reached the specified significant inclusion level. The first two splits considered MGS level; MGS 8-15 p showed 93 per cent 10-year survival in the largest group consisting of 127 out of 159 patients. In the remaining patients, with 16 MGS points or more, 44 per cent survived. There were 15 patients with 17 points and more, and the 10-year survival was only 27 per cent.

The MGS group of 8-15 points was split according to stage. Thus stage IA, IB and IIA gives 96 per cent survival, stage IIB only 72 per cent survival. In the latter group there were, however,

Table 9 a, b

Stepwise regression analysis of the 10-year survival/lethality (S/L) rate. Material: n=159.

Excluded patients: Dead within 5 years after diagnosis of inter-current disease and irradiation treatment less than 60 per cent of the intended dose, uncomplete MGS registration or poor biopsy quality.

Dependent variable: Survival/lethality rate 10 years after diagnosis (survival = 1 lethality = 0).

Predictors a: Predictors listed in Table 2 including the malignancy grading system (MGS).

" b: The same as a, excluding the malignancy grading system (MGS).

Program: BMDP2R (Ref. Dixon 1981)

Table 9 a

Stepwise regression analysis of the 10-year S/L rate.

Predictor	Regression coefficient	Standard error	t	Beta coefficient
MGS	-0.09	0.01	-7.6***	-0.51

Intercept = 2.09 $R^2 = 0.28$, RSD = 0.32

Table 9 b

Stepwise regression analysis of the 10-year S/L rate (MGS excluded).

Predictor	Regression coefficient	Standard error	t	Beta coefficient
Stage IIB	-0.20	0.07	-2.87**	-0.20
Structure(P1)	-0.22	0.09	-2.37*	-0.17
Vascular invasion (P7)	-0.16	0.04	-4.60***	-0.32
Host-cellular response(P8)	-0.12	0.04	-3.23**	-0.23

Intercept = 1.55 $R^2 = 0.27$, RSD = 0.33

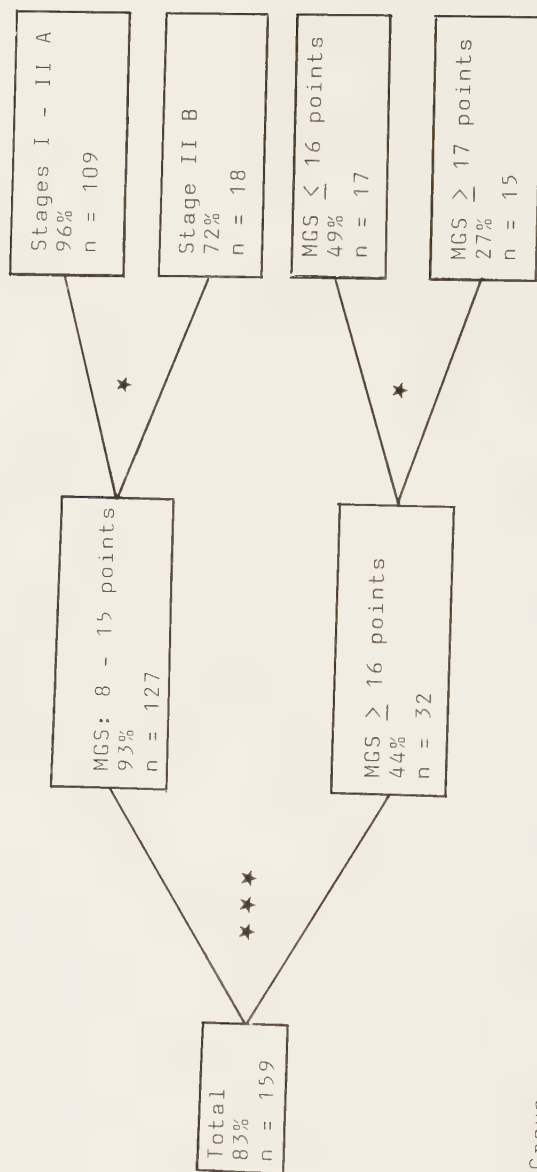
* Significant at 5% level

** " " 1% "

*** " " 1%o "

Figure 5

AID analysis of 10-year survival rates, n = 159.



only 18 patients. Other factors were not included in the AID tree because they did not reach the mass-significant criterion or were too small. AID analysis as well as regression analysis proved the dominating prognostic capacity of the MGS for 10-year survival. From the AID tree, a low risk patient group with 96 per cent 10-year survival could be identified, namely, patients with 15 or fewer points or in stages I A - II A.

The relationship between MGS and survival at e.g. 3, 5 and 7 years was also studied. It could be expected that since the MGS was determined at the time of admission the MGS importance measured as the proportional hazard rate will decrease successively with time. To study this question Cox proportional hazard regression model was used (Table 10).

Three analyses have been performed. The first one (A) included MGS alone. The second (Tables 10 B and C) included the combination of MGS and time (Z). The Z variable is defined in order to show change in the prognostic significance of the MGS over time. A positive Z means that the proportional hazard rate increases with the years after diagnosis. But a negative value means that the MGS importance decreases with the increased time interval from diagnosis. The regression analysis, considering even the censored cases, included 168 patients. An additional analysis including the above mentioned variables and P_7 (vascular invasion) and P_8 (tumour-host cellular response), was carried out (Table 10 B). The analysis shows that the MGS was the dominating variable (Table 10). No other single variable was significant. The coefficient corresponding to Z is positive, indicating that the importance of the MGS increases with time. This finding, even though not statistically significant, is surprising, probably corroborating with the fact that the lethality in carcinoma was higher during the first 5 years than during the latter years of the 10-year period. The coefficients given in Table 10 correspond to proportional hazard rates and illustrate the relative importance of the MGS. A coefficient of 0.54 was obtained for the MGS, disregarding the other variables, and was strongly significant. The coefficient 0.54 implies that

Table 10

Cox regression analysis with respect to survival.

Material: 168 patients.

Program BMDPL2.

Predictors: A. MGS alone

B. MGS, P_7 , P_8 and Z. Z is a time dependent variable, T (months) interacting with MGS. The constant 14 corresponds to mean MGS and the constant 4.1 to $\ln 60$.

C. MGS and Z.

Predictor	Coefficient	SE	t
A. MGS	0.538	0.077	7.0 ***
B. MGS	0.548	0.167	3.28**
P_7	0.309	0.315	0.98
P_8	0.448	0.294	1.52
$Z=(MGS-14)(\ln T-4.1)$	0.135	0.108	1.25
C. MGS	0.702	0.139	5.0
Z	0.150	0.107	1.40

The coefficients in Cox regression analysis correspond proportional hazard rates. (The significance asterisk as in previous tables).

an increase in the MGS of one point raises the lethality risk to a hazard rate of 71%. A two point increase in the MGS would correspond to an approximately three-fold risk of death in cancer ($1.71^2 \approx 3$). The estimates are, however, very uncertain. The Cox regression analyses performed are in agreement with the analyses carried out on the 10-year material and confirm the previous impression that MGS is the dominating prognostic factor.

Table 11
FIGO stage.

MGS	I A	I B	II A	II B	Total
<12	0/19	0/13	0/ 5	0/ 0	0/ 37
13-15	0/ 4	2/35	2/33	5/18	9/ 90
16>	0/ 0	5/10	10/15	3/ 7	18/ 32
Total	0/23	7/58	12/53	8/25	27/159

The ratio between the number of patients dead in the cancer disease at 10-year follow-up and the initial number (n=159) of patients in combination between MGS and FIGO stages. The table is the basis for the comparison between the correlations to S/L for MGS and FIGO stages (see the first line in Table 12).

Superiority of MGS

The ratio between the number of patients dead in cancer disease and the initial number of patients in combination between MGS and FIGO stages is given in Table 11. The table is the basis for the comparison between the correlations to S/L for MGS and FIGO stages (see the first line in Table 12). In table 12 the differences between correlations are given. The table shows that the correlation between MGS and S/L (S=0, L=1) is significantly stronger than other correlations to S/L. The significance

Table 12

Correlation between MGS and S/L at 10 year compared to the correlation between various variable (X) and S/L and the t-value for the difference (n=159, at most). MGS classified as <12 points, 13-15 p, 16 $\geq$ p, given value 1, 2 and 3 points respectively. The figures within brackets correspond to further subdivision of the 16 $\geq$ p group; 16 p given value 3 and 17 p $\geq$ given value 4.

Variable (x)	code	Difference		t
		r MGS, S/L	- r X, S/L	
Stage	IA, IB, IIA, IIB	0.22		2.53*
Differentiation into cell type (Reagan & Wentz)	Large cell non- keratinized. Large cell keratinized. Small cell carcinoma.	0.38		4.40***
Classification Ackerman & Del Regato n=128	High different. Moderate different. Anaplastic carcinoma.	0.20 (0.25)		1.95 (2.28*)
Age	10 years groups	0.40		4.19***
Admission year	1969, 1970	0.46+		3.92***
	P1	0.22		2.00*
	P2	0.25		3.19**
	P3	0.36		4.01***
	P4	0.44		4.34***
	P5	0.34		3.93***
	P6	0.19		2.78**
	P7	0.11 (0.17)		1.63 (2.38*)
	P8	0.15 (0.21)		1.72 (2.31*)

+ Because $r_{S/L} < 0$, ($r_{MGS, S/L}$) - ($r_{x, S/L}$) was constructed.

* 0.01 $< p \leq 0.05$

** 0.001 $< p \leq 0.01$

*** $p \leq 0.001$

analyses are performed as in Stendahl et coll. (1981 a) show that one half of the differences are highly significant ($P < 0.001$). Some, however, do not reach the 5% significance level. Thus, for histological classification according to Ackerman & Del Regato, vascular invasion (P_7) and host-cellular response (P_8), the correlations to S/L are lower than the correlation between MGS and S/L, but the correlation differences may be due to chance. However, after a small modification of the MGS classification (see the brackets in Table 12) significant ($p < 0.05$) differences are obtained even for these predictors. Thus, the superior predictive value of MGS is evident from the table. The superiority of the MGS in relation to clinical stage, illustrated in Table 11 and 12 is of special interest.

DISCUSSION

The results of this study are compatible with earlier results from a similar material (Stendahl et coll. 1979, 1983). The follow up of our material was extended to 10 years and confirms that even the long term prognosis was very well correlated to the MGS values.

The low MGS point patient group has an extremely good prognosis. This group represented 1/4 of the patients in our study. If the patients with the low point and the moderate point score are aggregated, 93% of patients were alive at the 10-year follow up, 95% confidence interval (87%, 97%). The aggregated group represents 4/5 of the patients in the study.

Still there is the high point group of patients (32 out of 159) with worse and difficult to predict prognosis according to the AID-tree. The explanatory values obtained by regression analyses are not particularly high and the similar results appeared in the AID analysis. It is important to find an explanation why in one-fifth of patients, the prognosis remains difficult to

predict. As for patients with very high scores it is known that the prognosis is unfavourable. In the present material the high lethality among the few cases with 17 points or more, is consistant with these earlier findings.

Before the MGS was included in the analyses each factor was studied separately with respect to the 10-year prognosis. Some factors showed an evidently significant result. However, these significant results did not remain after the MGS was included.

The superiority of the MGS was expressed by a comparison of the correlation between the MGS and the 10-year survival and the correlation reached by the other factors. The correlation between the MGS and the survival is highly significant, much higher than the correlation to each single factor and without exceptions the differences are significant. Thus, the MGS showed a stronger correlation to the 10-year survival than clinical stage according to FIGO when stage I A - II B were studied. This study confirms previous results (Stendahl et coll. 1979, 1981 a, 1983) that the MGS is superior to the established clinical FIGO staging in the prediction of outcome. With respect to predictive power it can be discussed if the MGS classification could be regarded as a suitable substitute for the FIGO staging I - III. This study limited to stage I A - II B (n = 168) as well as Stendahl's et al (1983) study of stages I B - III (n = 338) give support to this suggestion. Using both the malignancy grading system and clinical staging together may allow a somewhat better prediction of the clinical outcome in individual patients as indicated by the AID analysis; even if MGS is superior, the staging seems to have an additional predictive capacity.

The survival analysis shows that the lethality during the first 5-year period is considerably higher than during the second half of the 10-year period. It was possible to determinate if the prognostic capability of the MGS remains even in this later period. The analysis showed no sign of a time dependent decrease in the MGS prognostication capacity, measured as proportional

hazard effect.

Only a few pathologists have been involved in the MGS grading. In spite of the registration problems that have been noticed (Stendahl et coll. 1981 b), the high survival rates in groups with low and moderate MGS score is very promising. The present study as well as earlier studies are based upon a retrospective material only. By an improvement in the method of fixation and biopsy techniques, the estimating of the MGS items becomes more reliable (Willén et coll. 1983).

The original 198 cases had to be reduced to 168 cases due to a poor biopsy quality: Thirty patients were excluded because of incomplete registration of one or several items. Parameters for the tumour cell population were almost always possible to evaluate. Items for the tumour-host relationship, especially stage of invasion (P_6) and vascular invasion (P_7) were often missing. As the most important parameters were lacking, it was considered inadequate to base the MGS on remaining items.

In 31 cases, included among the 168 ones, the biopsy was assessed to be doubtful representative. The correlation between MGS and the outcome was, however, of the same magnitude among the doubtful cases as among the others.

Ten uncomplete treated cases were excluded. The limit was set at 60 per cent of the estimated irradiation dose in order to eliminate patients with expected unfavourable prognosis. In remaining cases, a non-significant correlation between dose of irradiation and MGS as predictor was observed. The setting of the exclusion limit was therefore a suitable one.

The comparison of the MGS and histopathologic staging according to Ackerman & Del Regato is limited by the fact that the classification was done by many different pathologists and a considerable proportion of cases was not classified in the primary histopathologic report. Only cases with both MGS grading and

Ackerman & Del Regato classification could be compared.

We conclude that the MGS system has a superior prognostic capacity in non-generalized invasive squamous cell carcinoma of the uterine cervix.

SUMMARY

168 patients with invasive squamous cell carcinoma of the uterine cervix stage IA - IIB treated by radiotherapy during the period 1969-1970 were studied in order to evaluate the correlation between certain prognostic factors and the 10-year survival /lethality rate. These factors included a malignancy grading system (MGS) consisting of 8 items; structure (P_1), differentiation into cell type (P_2), nuclear polymorphism (P_3), mitosis (P_4), mode of invasion (P_5), stage of invasion (P_6), vascular invasion (P_7), lymphoplasmocytic response (P_8). Also studied were histological differentiation according to Ackerman & Del Regato and differentiation into cell type (Reagan and Wentz), patients age, admission year, clinical stage according to the FIGO classification and irradiation.

Many of these factors were correlated to the prognosis. However, the MGS system was superior as predictive factor. Patients with a low MGS score had an extremely good survival rate at both the 5- and 10-year control. The patients with a high MGS score had an approximately 55 per cent lethality by 10 years. The MGS was significantly superior to each separate item as well as to each other predictive factor ($p \leq 0.05$). Further, no other important predictive factor could be identified after MGS had been included in the multivariate analyses. After the MGS, vascular invasion (P_7) and then cellular response (P_8), followed by the mode of invasion (P_5) and stage II B seemed to be the most important predictors. In stages I A - II B the MGS alone showed a better prognostic capacity than staging according to the FIGO classification alone. Our MGS grading results are compatible with previous similar studies and show that they are convenient even for the estimation of the 10-year survival.

REFERENCES

- ACKERMAN L.V. and DEL REGATO J.A.: Cancer diagnosis, treatment and prognosis. First edition. C.V. Mosby, St Louis, 1947.
- DIXON W.J. (Editor): BMDP. Statistical Software. University of California Press, Berkely, Calif. 1981.
- EKLUND G. and GAVATIN A.: Automatic interaction detector (AID) analysis. In: On the role of viruses in acute infectious diseases of the central nervous system. Edited by B. Sköldberg. Scand. J. infect. Dis. (1972). Suppl. No. 3, p. 89.
- FIGO - Fédération Internationale de Gynécologie et Obstétrique: Classification and staging of malignant tumours in the female pelvis. Acta obstet. gynec. scand. 50 (1971), 1.
- FRICK H.C., JONOSKI N.A., GUSBERG S.B. and TAYLOR H.C.: Early invasive cancer of the cervix. Am. J. Obstet. Gynec. 85 (1963), 100.
- JOHNSSON J.E. and NORDBERG U.B.: Dosimetric problems with radium in the intracavitary treatment of carcinoma of the uterine cervix. Acta radiol. Ther. Phys. Biol. 12 (1973) 257.
- JOHNSSON J.E.: Squamous cell carcinoma of the uterine cervix. Acta radiol. Ther. Phys. Biol. 16 (1977), 33.
- KOTTMEIER H.L.: Surgical and radiation treatment of carcinoma of the uterine cervix. Experience by the current individualized Stockholm technique. Acta obstet. gynec. scand. 43 (1964) Suppl. No. 2.
- OSIRIS III. An integrated collection of computer programs for the management and analysis of social science data. Ann Arbor, Michigan, 1973.

RATTENBURY J. and PELLETIER P.: Data processing in the social sciences with osiris. The University of Michigan, Ann Arborg, Michigan 48106, 1974.

REAGAN J.W. and WENTZ W.B.: Genesis of carcinoma of uterine cervix. Clin. Obstet. Gyneac. 10 (1967), 883.

Statistical abstract of Sweden. Official statistics of Sweden, National Central Bureau of Statistics, Stockholm, 64 (1977), 80.

STENDAHL U., EKLUND G., WILLÉN H. and WILLÉN R. (a): Invasive squamous cell carcinoma of the uterine cervix. III. A malignancy grading system for indication of prognosis after radiation therapy. Acta radiol. Oncology 20 (1981), 231.

STENDAHL U., EKLUND G. and WILLÉN R.: Prognosis of invasive squamous cell carcinoma of the uterine cervix: a comparative study of the predictive values of clinical staging I B - III and a histopathologic malignancy grading system. Int. J. Gyn. Path. 2 (1983), 42.

STENDAHL U., WILLÉN H. and WILLÉN R.: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. Acta radiol. Oncology 18 (1979), 481.

---Invasive squamous cell carcinoma of the uterine cervix. I. Definition of parameters in a histopathologic malignancy grading system. Acta radiol. Oncology 19 (1980), 467.

---:(b)Invasive squamous cell carcinoma of the uterine cervix. II. Reproducibility of a histopathologic malignancy grading system. Acta Radiol. 20 (1981), 65.

WILLÉN H., WILLÉN R. and STENDAHL U: Invasive squamous cell carcinoma of the uterine cervix. VII. Influence of vehicle osmolarity on biopsy quality. Acta radiol. Oncology 22 (1983), 257.

INVASIVE SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX

Construction of a partial index in a histopathologic malignancy grading system

Helena Willén* and Gunnar Eklund

Department of Pathology, University Hospital, Lund* and
Department of Cancer Epidemiology, Radiumhemmet, Karolinska
Hospital, Stockholm, Sweden.

Reprint request to: Dr Helena Willén
Department of Pathology
University Hospital
S-221 85 LUND Sweden

Acknowledgement: Financial support was received from the
University and University Hospital, Lund and John and Augusta
Persson's Foundation.

INTRODUCTION

A histopathologic malignancy grading system (MGS) for carcinoma of the uterine cervix has previously been introduced (STENDAHL et coll. 1979, 1980).

Several retrospective reviews have shown that total malignancy score is correlated to survival/lethality (STENDAHL et coll. 1979, 1981 b, 1983; EKLUND et coll. to be published).

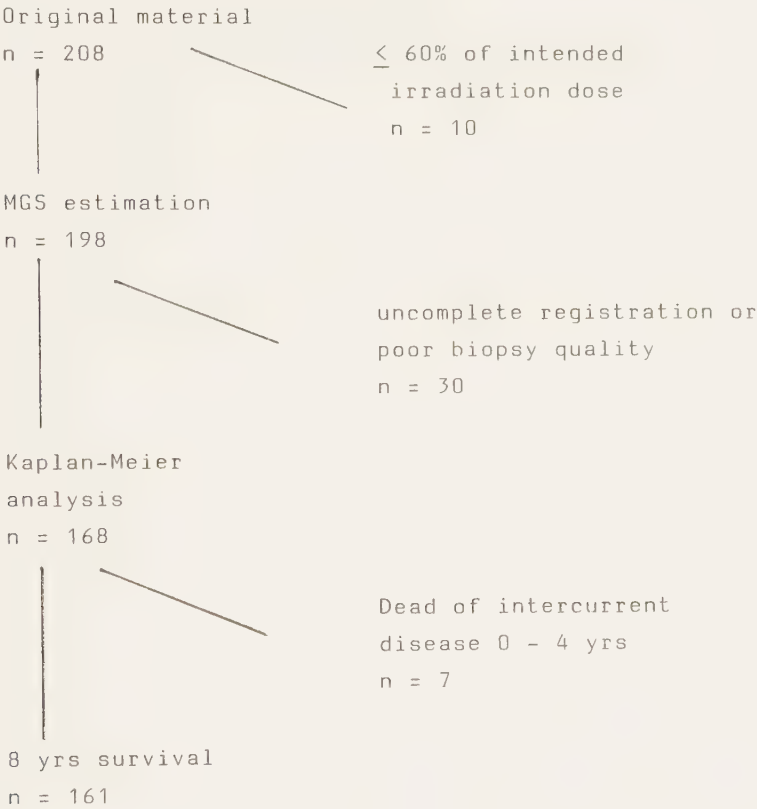
In earlier studies, a partial index, where only certain parameters are considered, is recommended (STENDAHL et coll. 1981 c). The reliability of such a partial index should be tested in further studies to assure that the parameters chosen in the original studies as having a high predictive value were not favoured by chance. Based on this reevaluation a new partial index is proposed.

MATERIAL

The material consisted of 208 patients with invasive squamous cell carcinoma of the uterine cervix stages I A to II B (FIGO 1971) treated at the Division of Gynecologic Oncology, Department of Oncology, University Hospital, Lund during the years 1969 to 1970. The boundary between stage I A and I B in cases where cone biopsy was performed has been established according to Frick et coll. (1963). The follow-up period was at least 10 years, but we give an account for only the first 8 year period. This has been done to make a comparison with a previous study possible (STENDAHL et coll. 1981 c). Patients who had received less than 60 per cent of the intended irradiation dose at point A were excluded (10 patients). No attention was otherwise paid to the tumour dose. Patients who had received external irradiation exclusively were included in the study. The material was reduced to 198 patients (Table 1).

Table 1

Patients with invasive squamous cell carcinoma of the uterine cervix included in this study, n = 161 and 168 respectively.



When the histopathologic malignancy grading was retrospectively performed, 30 patients were expelled who could not be evaluated with respect to all histologic parameters or had a biopsy of poor quality (leaving 168 patients).

Patients who died from intercurrent disease within 4 years after diagnosis of disease were also excluded, leaving 161 patients in the study.

Stage distribution of the material is presented in Table 2.

Table 2

Distribution of patients (8 yrs) by clinical stages, n = 161.

I A	23
I B	59
II A	53
II B	26
Total	161

METHODS

The grading of the histopathologic parameters was performed retrospectively on pretreatment biopsies, without knowledge of treatment and the further course of the disease.

The tumour-cell population parameters, namely structure (P_1), differentiation into cell type (P_2), nuclear polymorphism (P_3), mitoses (P_4), and the tumour-host relationship parameters, mode of invasion (P_5), stage of invasion (P_6), vascular invasion (P_7) and host cellular response (P_8), were recorded separately. Each parameter was graded from 1 to 3 points. Thus, after summing up the parameter values the score (MGS) permitted a grading from 8 to 24 points totally. The

distributions of the parameters and the MGS are given in EKLUND et coll.

The predictive values of the malignancy grading system and a partial index were compared with each other on this material with the aid of correlation and stepwise linear multiple regression analyses, BMDP2R, life table analysis according to Kaplan & Meier, BMDPL1 (DIXON 1981).

Table 3

Product-moment correlations between different histopathologic parameters and survival/lethality (S=0/L=1) after 8 yrs and the MGS.

Variable	S/L at 8 yrs	MGS
P1 structure	.226**	.286***
P2 differentiation into celltype	.193*	.421***
P3 nuclear polymorphism	.125	.355***
P4 mitoses	.022	.139
P5 mode of invasion	.155*	.431***
P6 stage of invasion	.265***	.635***
P7 vascular invasion	.404***	.626***
P8 host-cellular response	.341***	.426***
MGS	.516***	

* significant at 5% level

*** " " 1% " "

RESULTS

The correlations between each of the histopathologic parameters and S/L, S coded 0, L coded 1, as well as MGS are given in Table 3. The highest correlations to S/L is reached by MGS followed by

vascular invasion (P_7), host-cellular response (P_8) and stage of invasion (P_6). With respect to rank those findings were in total accordance with previous results (STENDAHL et coll. 1981 c).

The strenght of the correlations were also similar in both materials. There was, however, a significant difference in between the correlations between P_5 (mode of invasion) and survival. The correlation coefficient in our material for this variable was 0.155 while in the Uppsala study it was 0.392.

All parameters, except P_4 (mitoses) showed a highly significant correlation to MGS ($p < 0.001$).

Table 4

Correlation coefficients, r , between the eight items included in the MGS.

	P1	P2	P3	P4	P5	P6	P7	P8
P1	1.00							
P2	.19	1.00						
P3	.00	-.04	1.00					
P4	-.28	.06	-.16	1.00				
P5	.07	.10	.05	-.10	1.00			
P6	.16	.15	.03	.08	.22	1.00		
P7	.10	.19	.03	.01	.22	.25	1.00	
P8	.26	.03	.01	-.29	.00	.21	.17	1.00

Significance: $p < 0.05$ corresponds to $r \geq 0.16$

$p < 0.01$ " " $r \geq 0.20$

$p < 0.001$ " " $r \geq 0.26$

Table 4 shows the correlations between pairs of the eight items included in the MGS. The correlations were generally quite low.

Particularly striking were the negative correlations between mitoses (P_4) and the other variables. The correlation within the tumour-cell population variables ($P_1 - P_4$) was poor throughout. Among the tumour-host relationship ($P_5 - P_8$) variables, correlation was somewhat higher, but still quite low. When the correlation table was considered, it did not appear feasible to eliminate any of the items because of the low correlation between them. It would mean that the parameters constituting the MGS are relatively independent. A multiple stepwise linear regression analysis was performed (Table 5) to study the possibility of reducing the number of parameters. Vascular invasion (P_7) followed by host-cellular response (P_8) exhibited strong significance, $P < 0.001$, but no other parameters reached the inclusion level.

Table 5

Stepwise multiple regression analysis.
Dependent: Survival/lethality (0/1 code) at 8 year control.
Independent: $P_1, P_2, P_3, P_4, P_5, P_6, P_7$, and P_8 .
Inclusion level: $t \geq 2.0$ corresponding to $p \leq 0.05$.
Program: BMDP 2R, 1981.
Material: 161 cases.

Variable in equation	Regression coefficient	Standard error
P7 vascular invasion	0.170***	0.034
P8 host-cellular response	0.138***	0.035

$P_1 - P_6$ candidated, but were not included
Multiple R = 0.489.
Order of inclusion: P_7, P_8 .
Significance marked as in Table 3.

Table 6

Correlation between, on one hand different item combinations, and on the other S/L (8 year) and the MGS.

	S/L 8 yrs	MGS
Combination	n = 161	n = 161
	r	r
P1+P7	444	665
P5+P7	379	690
P7+P8	487	689
P5+P7+P8	474	766
P1+P5+P7	414	721
P1+P5+P7+P8	493	774
P4+P5+P7+P8	476	813
P1+P2+P3+P4	252**	610
P5+P6+P7+P8	483	856
2xP7+P5+P8	480	761
2xP7+P1+P5+P8	495	773
2xP7+P4+P5+P8#	481	796
1.235xP7+P8##	489	699

** Significant at 1% level

All other correlations significant at 1% level

Partial index according to Stendahl et coll. (1981)

Weights proportional to regression coefficient in table 5
(1.235 = 0.170/0.138).

The results indicate that it would be possible to reduce the number of variables considerably without loss of predictive capacity. In order to illustrate the strength of various simple combinations of the parameters, they have been subjected to a correlation analysis (Table 6).

Among the combinations made by four items, the parameters representing the tumour-cell population ($P_1 - P_4$) were relatively poorly correlated to S/L. The tumour-host relationship ($P_5 - P_8$) showed a considerably higher correlation to both S/L and MGS. The index recommended by STENDAHL et coll., which includes mitoses, was not the most successful but not significantly inferior to the seemingly best combination. Thus, combinations of the tumour-host parameters showed highest correlations (Table 6).

The partial index based on a combination of two items only (2 - 6 points), vascular invasion and host-cellular response ($P_7 + P_8$), could identify low and high risk patients, (Table 7). The table is the basis for a comparison between the correlations to S/L for $P_7 + P_8$ and FIGO stages. The correlation is significantly stronger ($p < 0.05$) for $P_7 + P_8$ than for stages I A - II B. The difference between the correlations is 0.23 (0.49 - 0.26). This simple combination $P_7 + P_8$ can give as good correlation to S/L as the best index composed of three or four items. Comparison between the survival curves for $P_7 + P_8$ (Figure 1) and a MGS classification (Figure 2) reveals that the simple combination $P_7 + P_8$ (vascular invasion and host-cellular response) seem to have the same predictive capacity as MGS. In the figures 1 and 2, the materials are divided into three patient groups.

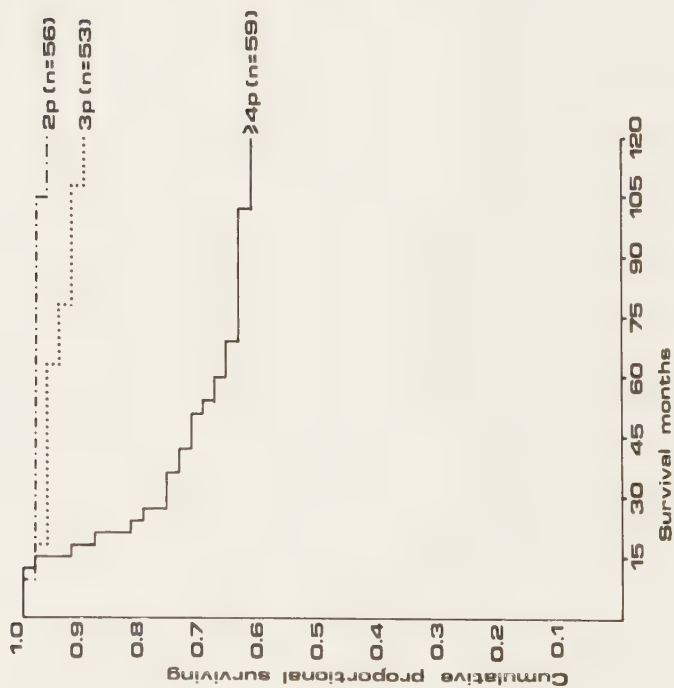


Fig 1.

Survival curves for the partial index based on $P_7 + P_8$, ranged 2 to 6 points.

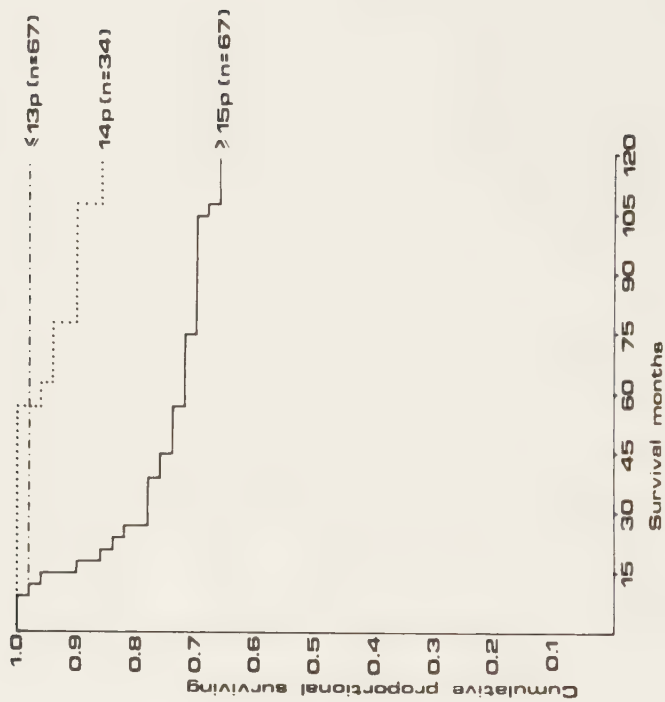


Fig 2.

Survival curves for three MGS classes.

Table 7

Partial index 2 - 6 points P7 + P8 (vascular invasion and host-cellular response) and clinical staging I A - II B (FIGO) qualified 1 - 4 compared to survival/(survival+lethality) in 161 patients.

P7+P8 (points)	1 I A	2 I B	3 II A	4 II B	Total (% survivals)	
2(min.)	16/16	19/19	11/11	7/8	53/54	(98)
3	5/5	22/22	13/14	7/10	47/51	(92)
4	2/2	6/8	15/17	4/6	27/33	(82)
5		6/7	2/8	1/2	9/17	(53)
6(max.)		1/3	0/3		1/6	(17)
	23/23	54/59	41/53	19/26	137/161	
(% survivals)	(100)	(92)	(77)	(73)	(85)	

DISCUSSION

The collection of information by use of qualitative and quantitative parameters requires analysis of the prognostic values of each parameter and all combinations together. Generally, parameters chosen for partial index should be easy to evaluate, correlate to survival as well as to the MGS, and add further prognostic information.

Previous study on the reproducibility of a histopathologic malignancy grading system (STENDAHL et coll. 1981 a) showed that some parameters were more difficult to evaluate than others. Structure, cell type, vascular invasion and cellular response were easier to evaluate compared to nuclear polymorphism, mitoses, mode of invasion and stage of invasion, which most often depend on biopsy quality and subjective factors associated

with the histopathologic evaluation. A histopathologic malignancy index proved to be able to discriminate patients with carcinoma of the uterine cervix with respect to the prognosis (STENDAHL et coll. 1981 b, 1983). Eight histopathologic items graded from 1 to 3 add up to constitute the malignancy index. By regression analysis the number of items could be reduced to 4: mitoses (P_4), mode of invasion (P_5), host-cellular response (P_8) and vascular invasion (P_7), the latter was attributed double weight. The predictive value of the partial index seemed to be as good as the malignancy index (MGS) itself. These calculations were obtained upon material from patients in stage II only and confirmed on a material in stage I, III and IV. In pilot studies, however, there is a risk that favourable combinations attract attention (STENDAHL et coll. 1981 c).

In the present study, 168 patients received an adequate complete treatment and had a biopsy of a proper quality for an evaluation of all histopathological parameters ($P_1 - P_8$). A considerable number of patients were excluded (see MATERIAL). One of the reasons for this was an uncomplete registration of different items, particularly stage of invasion (P_6), vascular invasion (P_7) and host-cellular response (P_8). In this study, 15 cases were excluded because of missing registration of P_6 , P_7 and P_8 . The question is if transition to a partial index can give a satisfactory prognostic information when some but not all items are registrated. From table 6 it is obvious that tumour-cell population items ($P_1 - P_4$) were less informative. A partial index not based on parameters of the tumour-host relationship (especially P_7 and P_8) will scarcely be of any success.

$P_1 - P_4$ items showed a weak correlation to S/L in this as well as in an earlier study (STENDAHL et coll. 1981 c). In particular, P_4 (mitoses) was of dubious worth and had no prognostic value by itself, but, nevertheless was included in their index. Our analysis supports the possibility that this item has been favoured by chance. Assessments of mitotic

activity are often used in routine surgical pathology (SILVERBERG 1976). Among factors with influence upon assessment can be a delay in fixation, which can allow the majority of Mitosis to terminate, resulting in unreliable evaluation of mitotic activity (GRAEM and HELWEG-LARSEN, 1977). The estimated number of mitoses depends on the section thickness, as well (ELLIS and WHITEHEAD, 1981). CHI et coll. (1977) found variable frequency and distribution of mitotic figures within the different tissue layers in dysplasia and carcinoma in situ of the uterine cervix. Still, there may be an underestimation of the mitosis parameter value in the study of invasive squamous cell carcinoma of the uterine cervix.

There are some differences in the composition of our material and the Uppsala material, the latter being composed of the more advanced cases. Our material was limited to stage I A - II B, which resulted in a lower MGS score. Our reduced index may be less successful when applied to stage III cancers.

In our study, the partial index based on parameters representing the tumour-host relationship ($P_5 - P_8$) showed a strong correlation to S/L and MGS ($P < 0.001$).

Mode of invasion (P_5) was included as a second parameter in the regression analysis of the Uppsala material but not at all in our study. It should be noted that a score of three for P_5 was much less common in our material ($n = 10$) than in Uppsala's ($n = 40$) and that may explain the lower correlation. As the material in the present study had a different stage composition and was dominated by cases with a low score, the lower S/L correlation of our material compared to the Uppsala one was not surprising. Besides the lethality at 8-year follow-up was considerably lower in the Lund material.

Vascular invasion has proved to be a valuable prognostic parameter in this, as well as in an earlier study (STENDAHL et coll. 1981 c). A possibility of an interobserver error and technical

errors must nevertheless be considered. Shrinkage or fragmentation of the biopsy specimen can give spaces with intima-like covering. That is the case especially in a retrospective, inadequately fixed material. The influence of different fixative methods on vascular invasion estimation has been studied by WILLEN et coll. (1983). With an improved fixation, there is a minimal tissue shrinkage and sectioning artefacts. Vessels remained open which facilitates the analysis of vascular invasion.

It seems to be important to include parameters estimating the host-cellular response in the partial index which showed a highly significant correlation to S/L as well as the MGS. The evaluation and reproducibility of two parameters P_7 and P_8 are easier if the biopsy quality is acceptable and the definition of the parameter are consistently applied. The cellular response was very intense in the group of patients without deaths and recurrences and seems to be an indication for a low death risk, though the exact mechanism involved is not known. A beneficial effect of this factor on prognosis has now been reported for squamous cell carcinoma of the uterine cervix (BALTZER et coll. 1982) as well as a wide spectrum of neoplasms (KHOO and MACKAY, 1974).

Concerning the possibility to reduce the number of items, the results of the present study are in agreement with those of the Uppsala material (STENDAHL et coll. 1981 c). Still, we find several reasons for the use of a new partial index. It is possible to obtain a good prediction of prognosis with fewer than 8 items. Summing up the Uppsala and Lund material, the original partial index ($P_4 + P_5 + 2 P_7 + P_8$) still remains as the combination which shows the highest correlation to the S/L mean. On the other hand, the more reliable tumour-host relationship ($P_5 + P_6 + P_7 + P_8$) was comparable with that original partial index. Even further reduction to only three items ($P_5 + P_7 + P_8$) gave a good correlation to MGS and S/L. A surprisingly good correlation to S/L was obtained

when only two items, $P_7 + P_8$ (vascular invasion and cellular response) were used (demonstrated in Fig 1, 2). An index based on $P_7 + P_8$ showed a significantly stronger correlation to the outcome than the FIGO classification in stages I A - II B. The same superiority has earlier been observed for MGS (EKLUND et coll.).

Overly complicated and time-consuming systems of classification are a well known problem in surgical pathology. Poor reproducibility and training problems limit the use of the MGS to a small exclusive group of superspecialized squamous cell carcinoma experts or gynecologic pathologists (STENDAHL et coll. 1981 a).

The reduction of the histological items to the estimation of tumour-host relationship and particularly to $P_7 + P_8$ (vascular invasion and host-cellular response) can open a new discussion for using this classification in routine diagnostic practice. A new reproducibility study of MGS and of a partial index with only the $P_7 + P_8$ items on a prospective material using both traditional and improved fixative method is in progress in order to further evaluate this problem. Nevertheless, MGS remains for this moment the most complete and competent prognostic classification for the invasive squamous cell carcinoma of the uterine cervix.

SUMMARY

A malignancy grading system (MGS) for invasive squamous cell carcinoma of the uterine cervix consisting of four items for the tumour-cell population and four items for the tumour-host relationship has been proved to have a good prognostic capacity with long follow-up. A correlation analysis based on 161 patients with invasive squamous cell carcinoma of the uterine cervix in stage I and II has been evaluated. Relationship between S/L rate and all single items, MGS, and a reduced partial index have been compared. There was a good correlation

between the present study and previous results of another partial index. MGS has the best predictive value, but it is possible to reduce eight items to four (e.g. items for the tumour-host relationship) or to the items vascular invasion and host-cellular response ($P_7 + P_8$) alone, without any substantial loss of predictive capacity. The items of the tumour-cell population had no significant predictive value alone.

For practical reasons it seems to be motivated to propose and test a new partial index limited to $P_7 + P_8$ (vascular invasion and host-cellular response) only,

REFERENCES

- BALTZER J., LOHE K.J., KÖPKE W. and ZANDER J: Histological criteria for the prognosis in patients with operated squamous cell carcinoma of the cervix. *Gynec. Oncol.* 13 (1982), 184.
- CHI C.H., RUBIO C.A. and LAGERLÖF B: The frequency and distribution of mitotic figures in dysplasia and carcinoma in situ. *Cancer* 39 (1977), 1218.
- DIXON W.J. (Editor): BMDP. Statistical software, 1981. University of California Press, Berkely, Calif. 1981.
- EKLUND G., JOHNSON J.-E., STENDAHL U., TROPÉ C. and WILLÉN H.: Survival and malignancy grading of squamous cell carcinoma of the uterine cervix treated by irradiation (to be published).
- ELLIS P.S.J. and WHITEHEAD R.: Mitosis counting - a need for reappraisal. *Hum. Path.* 12 (1981), 3.
- FIGO - Fédération Internationale de Gynécologie et Obstétrique: Classification and staging of malignant tumours in the female pelvis. *Acta obstet. gynec. scand.* 50 (1971), 1.
- FRICK H.C., JONOSKI N.A., GUSBERG S.B. and TAYLOR H.C.: Early invasive cancer of the cervix. *Am. J. Obstet. Gynec.* 85 (1963), 100.
- GRAEM N. and HELWEG-LARSEN K.: Mitotic activity and delay in fixation of tumour tissue. *Acta path.microbiol. scand. sect. A.* 87 (1979), 375.
- KHOO S. and MACKAY E.V.: Relation of cell-mediated immunity in woman with genital tract cancer to origin, histology and clinical stage and subsequent behaviour of neoplasm. *J. Obstet. Gynaecol. Br. Commonw.* 81 (1974), 229.

SILVERBERG S.G.: Reproducibility of the mitosis count in the histologic diagnosis of smooth muscle tumours of the uterus. Hum. Path. 7 (1976), 451.

STENDAHL U., WILLÉN H. and WILLÉN R.: Classification and grading of invasive squamous cell carcinoma of the uterine cervix. Acta radiol. Oncology 18 (1979), 481.

---Invasive squamous cell carcinoma of the uterine cervix. I. Definition of parameters in a histopathologic malignancy grading system. Acta radiol. Oncology 19 (1980), 467.

---(a) Invasive squamous cell carcinoma of the uterine cervix. II. Reproducibility of a histopathologic malignancy grading system. Acta radiol. Oncology 20 (1981), 65.

STENDAHL U., EKLUND G., WILLÉN H. and WILLÉN R.(b): Invasive squamous cell carcinoma of the uterine cervix. III. A malignancy grading system for indication of prognosis after radiation therapy. Acta radiol. Oncology 20 (1981), 231.

STENDAHL U., EKLUND G. and WILLÉN R.(c): Invasive squamous cell carcinoma of the uterine cervix. IV. Analysis of a histopathological malignancy grading system and construction of a partial index. Acta radiol. Oncology 20 (1981), 189.

STENDAHL U., EKLUND G. and WILLÉN R.: Prognosis of invasive squamous cell carcinoma of the uterine cervix: A comparative study of the predictive values of clinical staging I B - III and a histopathologic malignancy grading system. Int. J. Gyn. Path. 2 (1983), 42.

WILLÉN H., WILLÉN R. and STENDAHL U.: Invasive squamous cell carcinoma of the uterine cervix. VII. Influence of vehicle osmolarity on biopsy quality. Acta radiol. Oncology 22 (1983), 257.

